U.PORTO
FACULDADE DE CIÊNCIAS DA NUTRIÇÃO E ALIMENTAÇÃO
UNIVERSIDADE DO PORTO

U.PORTO
FACULDADE DE CIÊNCIAS
UNIVERSIDADE DO PORTO

Dietary and Nutritional Intake and Low-grade Inflammation in Adolescents: the LabMed Physical Activity Study

Juliana Almeida de Souza

Porto | 2016

Dietary and Nutritional Intake and Low-grade Inflammation in Adolescents: the LabMed Physical Activity Study

Juliana Almeida de Souza

Porto | 2016

Dissertação de candidatura ao grau de Doutor apresentada à Faculdade de Ciências da Nutrição e Alimentação.

Orientador: Professor Doutor Pedro Alexandre Afonso de Sousa Moreira

Faculdade de Ciências da Nutrição e Alimentação, Universidade do Porto, Portugal

Coorientadora: Professora Doutora Rute Marina Roberto dos Santos

Early Start Research Institute, University of Wollongong, Australia.

Centro de Investigação em Actividade Física, Saúde e Lazer, Faculdade de Desporto, Universidade do Porto, Portugal.

Manuscripts

Fazem parte desta dissertação os seguintes manuscritos submetidos para publicação:

I. Fat intake and low-grade inflammation in Portuguese adolescents from LabMed Physical Activity Study

II. 'Five-a-day' fruit and vegetable intake recommendation is negatively associated with low-grade inflammation in adolescents: the LabMed Physical Activity Study

III. Associations between fruit and vegetable variety and low-grade inflammation in Portuguese adolescents from LabMed Physical Activity Study

IV. Dietary inflammatory index and inflammatory biomarkers in adolescents from LabMed Physical Activity Study

Participei na recolha de dados do LabMed Physical Activity Study, nomeadamente nas medições antropométrica e aplicação de questionários, bem como na informatização dos dados, revisão e correção da base de dados do LabMed Physical Activity Study.

Colaborei na definição dos objetivos destas manuscritos, na análise e interpretação dos dados de todos os artigos.

Fui responsável pela redação das versões iniciais de todos os artigos e participei ativamente na elaboração das versões finais.

"Há duas coisas na vida que se você guardar, você perde:
***conhecimento** e **afeto**. Se você os guarda, eles vão embora.*
*A única maneira de ter **conhecimento** e **afeto** é partilhá-los"*

Mário Sérgio Cortella

Acknowledgments

Aos meus dois queridos orientadores, os meus mais sinceros agradecimentos! Não vos agradeço tanto pelo trabalho em si, porque esta tese também é vossa. Como dizia o poeta Gregório de Matos, "o todo sem a parte não é todo". Este projeto é o todo de três partes, que muito se dedicaram-se para tal. No entanto, eu vos agradeço muito pela forma como o fizeram. Muito obrigada! Ambos foram imprescindíveis para o meu desenvolvimento intelectual, foram e serão modelos inspiradores para a minha atividade profissional.

Caro professor Pedro, como é possível não gostar de si? Um ser humano tão gentil, tão meigo, tão cortês; e um profissional competente e dedicado. Pedir para ser orientada por si foi certamente a escolha mais acertada que eu fiz durante o doutoramento! Também foi um prazer muito grande trabalhar consigo, apreender consigo e ser conduzida durante todo o processo de escrita por si. Muito obrigada pela partilha de conhecimentos e pela amizade.

Querida Rute, que sorte eu tive de me cruzar contigo neste projeto, uma pessoa amiga e carinhosa, mas ao mesmo tempo dinâmica e eficiente! Muito obrigada por me ter recebido de braços abertos no LabMed Physical Activity Study, foi um desafio e uma experiência muita rica. Na última etapa deste processo resolveste embarcar numa grande aventura. E apesar de distante, estivesse sempre presente! Agradeço-te muito pela oportunidade de aprender contigo e pelo teu carinho.

Agradeço ao Instituto Politécnico de Bragança, na pessoa do seu presidente, Professor Doutor João Sobrinho Teixeira, e à Escola Superior de Saúde, na pessoa da sua diretora, Professora Doutora Helena Pimentel, por terem criado os contextos que proporcionaram em parte as condições para que eu me dedicasse à realização deste trabalho, nomeadamente por ter suportado o programa de formação avançada atribuído pela Fundação Portuguesa para a Ciência e Tecnologia (SFRH/PROTEC/68092/2010), bem como através da dispensa e da facilitação de alteração de carga horária letiva.

Não poderia deixar de agradecer também aos adolescentes e aos seus pais, aos professores e às escolas do LabMed Physical Activity Study, pela participação nas recolhas de dados e por todo o tempo dedicado a este estudo.

Reconheço também o papel precioso do Departamento de Higiene e Epidemiologia da Faculdade de Medicina da Universidade do Porto, na pessoa do seu Diretor, Professor Doutor Henrique Barros, que presta um serviço muito importante à comunidade científica que utiliza o seu questionário de frequência alimentar, fazendo a conversão dos dados do questionário em quantidade de energia, de nutrientes e de alimentos.

Agradeço ao Centro de Investigação em Actividade Física, Saúde e Lazer (CIAFEL) da Universidade do Porto, na pessoa do professor doutor Jorge Mota, por patrocinar o LabMed Physical Activity Study. O CIAFEL é apoiado pela Fundação Português para a Ciência e Tecnologia (UID/DTP/00617/2013).

Não poderia de deixar de reconhecer a cooperação de todos os voluntários do LabMed Physical Activity Study que foram determinantes pra que este projeto tenha tido um resultado positivo. O meu muito obrigado à Rute Santos, Luís Lopes, Carla Moreira, Sandra Abreu, César de Agostini, José Santos, Filipe Biscoito, Sónia Ferreira, Hélder Sousa e tantos outros.

Também estou muito grata às minhas colegas de trabalho que me deram um suporte valioso, em especial à Vera, Olívia, Carina e Luísa. Desde trocas de horários de aulas, a imensas substituições em vigilâncias; foram inúmeros afazeres do nosso dia-a-dia profissional em que estivésseis sempre prontas para me apoiar. Muito obrigada, meninas!

Agradeço também aos meus amigos Vera, Ovídio, Cláudia, Silvestre, Olívia, Isabel, Augusta, Fernando, António, Andreia, Gil e Tânia pelo carinho e apoio. Também não poderia deixar de agradecer à madrinha Madalena pela ajuda e preocupação.

Muito obrigada à minha família por todo o suporte e compreensão. Um especial obrigado à minha mãe Cristina e ao meu irmão Victor. Vieram diversas vezes à Portugal e eu me dediquei tão pouco tempo a vocês os dois, partilharmos muito menos tempo do que aquele que merecíamos. Por outro lado, foi uma oportunidade para estreitarem os laços com o Guilherme.

Um muito obrigado a todos os avós, tios e tias, amigos e amigas do Guilherme, por todo o vosso tempo que dedicaram a ele e me substituíram tantas vezes durante (sempre, mas principalmente) este último ano. Em especial, obrigada às avós e ao tio (Cristina, Conceição e Victor). Naturalmente que não o fizeram com pesar, mas sim com todo o prazer de partilharem bons momentos com o nosso menino, o que me deixou tranquila e segura para continuar este trabalho, para enfrentar a desafiante tarefa de ser mãe, esposa, trabalhadora e estudante.

Agradeço ao meu marido e ao meu filho, Rui e Guilherme, que são a minha razão de existir. Gui, filho, eu nunca vou esquecer de quando tu disseste chorando "a mamã está quase a acabar o doutoramento", depois de me ouvir dizer que "agora a mamã não pode brincar". Com apenas três aninhos já demonstraste compreensão, amor, carinho... apesar de sentir a dor da ausência da mamã. Muito obrigada, filho! Rui, estamos e estaremos juntos para relembrar estes momentos da nossa vida em que eu tive de estar um pouco ausente da nossa vida privada para me dedicar à vida profissional. Tu foste um impecável pai, muitas vezes mãe, sem deixar de ser o meu grande companheiro. Muito obrigada, Lindo!

Table of Contents

1. Abstract

Inflammation is a normal physiological host defense mechanism and an essential component of immunity. Nevertheless, a state of low-grade inflammation has been recognized as a pathological feature of a wide range of chronic conditions including obesity, metabolic syndrome, diabetes, and cardiovascular disease, and as playing a role in the early-stage pathophysiology of these conditions. However, the issue of causality and the degree to which inflammation contributes as a risk factor to the development of disease remain unclear.

Considering that the evidence suggests that many foods, nutrients, and other food components may modulate inflammation, the aims of this thesis were to study the associations between low-grade inflammation and several variables related to dietary and nutritional intakes, specifically, the consumption of several nutrients (fatty acids), fruits (including variety), vegetables (including variety and vegetable soup), and a dietary inflammatory index (DII) score in adolescents.

We conducted a cross-sectional study based on baseline data collected in 2011 from the LabMed and Physical Activity Study with a sample of 412 adolescents (14.9±1.72 years old), over half of them girls (52.4%). We evaluated low-grade inflammation measuring inflammatory biomarkers and dietary and nutritional intakes using a food-frequency questionnaire (FFQ), moderate-to-vigorous physical activity, and sedentary time using accelerometers, and body mass index using anthropometric measurements. Pubertal development, socioeconomic status, and smoking habits were self-reported.

Inflammatory biomarkers were measured in blood samples. The blood samples were collected under fasting conditions, refrigerated, and sent to a laboratory to determine C-reactive protein (CRP), interleukin 6 (IL-6), and complement components 3 (C3) and 4 (C4). We created categories of lower or higher (inflammatory state) for each biomarker, considering sex- and age-adjusted median values. An overall inflammatory biomarker score was designated by adding the inflammatory biomarkers in the categories; each category was assigned one point if the biomarker was above the sex- and age-adjusted median values or zero if it was below the median. The overall inflammatory biomarker score computed was classified as 0–1 or 2–4 biomarkers above the median (lower or higher inflammatory state).

Dietary and nutritional intakes were estimated using an FFQ and the nutritional analysis software Food Processor Plus. In each manuscript, we considered dietary and nutritional intake variables differently. In paper I, dietary fatty acids were measured as a percentage of total energy intake (%en) and categorized into tertiles. In paper II, we considered the consumption of fruits, vegetables, and vegetable soup in daily portions, and we created categories of <3 or ≥3 portions/day for fruits and for vegetables and <2 or ≥2 portions/day for vegetable soup. We assessed adherence to the "5-a-day" recommendation (a public health message to encourage individuals to consume five or more daily servings of fruits and vegetables), totaling the number of portions of fruits, vegetables, and vegetable soup, and creating categories of <1, 1–5, or ≥5 portions/day. In paper III, the consumption of a variety of fruits and vegetables was assessed considering the number of FFQ items of fruit or vegetable intake (items are individual and combined fruits or vegetables) "at least once a month" in categories/month, and the data were categorized into tertiles. In paper IV, the DII score was calculated based on 31 food parameters and categorized into tertiles.

Odds ratios (OR), 95% confidence intervals (95% CI), and p values for trend (p_{trend}) were calculated from binary logistic regression, adjusted for confounders, in order to estimate the magnitude of association between inflammatory biomarkers and the overall inflammatory biomarker score, as independent variables, and dietary and nutritional intake variables, as dependent variables. In all papers, confounders considered were sex, age, moderate-to-vigorous physical activity, sedentary time, body mass index, pubertal development, socioeconomic status, and smoking habits as well as total energy intake in papers II, III, and IV.

In paper I, we showed that adolescents with a higher consumption of saturated fatty acids (SFA) (≥11.1%en) compared to a lower consumption (≤9.7%en) had higher odds of having a higher level of IL-6 (OR=2.16, 95%IC: 1.02–4.56, p_{trend}=0.051) and a higher overall inflammatory biomarker score (OR=2.51, 95% IC: 1.20–5.27, p_{trend}=0.015). In addition, adolescents with a higher intake of α-linolenic acid (ALA) (≥0.50%en) and eicosapentaenoic plus docosahexaenoic acids (EPA+DHA) (≥0.22%en), when compared to adolescents with lower intake

(≤0.42%en and ≤0.12%en, respectively), had lower odds of having a higher overall inflammatory biomarker score (OR=0.49, 95% CI: 0.26-0.95, p_{trend}=0.047, and OR=0.46, 95% CI: 0.23–0.93, p_{trend}=0.025, respectively).

In paper II, we found that adolescents who consumed <2 portions/day of vegetable soup had higher odds of having a higher level of IL-6 (OR=12.95, 95% CI: 1.63–102.84, p<0.05) than adolescents who consumed ≥2 portions/day. In addition, adolescents with an intake of <1 portion/day, when compared to ≥5 portions/day of fruits and vegetables (5-a-day recommendation adherence), showed high odds of having a higher level of CRP (OR=5.24, 95% CI: 1.62–16.97, p_{trend}=0.005), IL-6 (OR=5.36, 95% CI: 1.64–17.53, p_{trend}=0.008), and C4 (OR=3.39, 95% CI: 1.12–10.28, p_{trend}=0.016) and a higher overall inflammatory biomarker score (OR=8.14, 95% CI: 2.44–27.18, p_{trend}<0.001).

In paper III, we showed that adolescents with a higher variety of vegetable consumption (≥13 categories/month) had lower odds of having a higher level of CRP (OR=0.31, 95% CI: 0.15–0.64, p_{trend}=0.004) compared to adolescents with lower-variety consumption (≤6 categories/month), independent of quantity of vegetable intake.

In paper IV, we found that adolescents classified in the third tertile of the DII score (high inflammatory-diet properties) compared to those in the first tertile (low inflammatory-diet properties) had higher odds of having a higher level of IL-6 (in all models: crude model, OR=1.88, 95% CI: 1.09–3.24, p_{trend}=0.011; after adjustment for sex, OR=1.84, 95% CI: 1.06–3.18, p_{trend}=0.015, and in the fully adjusted model, OR=3.38, 95% CI: 1.24–9.20, p_{trend}=0.023). In addition, adolescents with a higher DII score (third tertile) compared to those in the first tertile had higher odds of having a higher level of C4 (0R=3.12, 95% CI: 1.21–8.10, p_{trend}=0.016) and a higher overall inflammatory biomarker score (OR=5.61, 95% CI: 2.00–15.78, p_{trend}=0.002) in the fully adjusted model.

In summary, low-grade inflammation was positively associated with the intake of SFA and negatively associated with intakes of ALA, EPA+DHA, vegetable soup, 5-a-day recommendation adherence, vegetable variety, and an anti-inflammatory dietary pattern.

The findings of this thesis support the recommendations for adolescents to limit SFA intake and to increase n-3 polyunsaturated fatty-acid intake, especially ALA, EPA, and DHA, and the consumption of two portions/day of vegetable soup, adherence to the 5-a-day recommendation, and eating a variety of vegetables. Furthermore, the DII can be a useful tool to assess a diet's inflammatory potential for adolescents and its association with low-grade inflammation.

2. Resumo

A inflamação é um processo fisiológico normal de defesa do organismo e um componente essencial da imunidade. Contudo, a inflamação de baixo grau tem sido reconhecida com uma característica patológica de uma série de condições crónicas como a obesidade, a síndrome metabólica, a diabetes e as doenças cardiovasculares, e como tendo um papel na fisiopatologia destas condições em estádios mais precoces. No entanto, a questão relativa à causalidade e ao grau em que a inflamação contribui como um fator de risco de desenvolvimento de doenças ainda não são claros.

Considerando que a evidência sugere que muitos alimentos, nutrientes e outros componentes alimentares podem modular a inflamação, os objetivos considerados para esta tese foram estudar a associação entre a inflamação de baixo grau e diversas variáveis relacionadas com a ingestão alimentar e nutricional, nomeadamente o consumo de alguns nutrientes (ácidos gordos), de frutas (incluindo a variedade) e de hortícolas (incluindo a variedade e a sopa), bem como a pontuação de um índice inflamatório da alimentação (foi utilizado o DII – *dietary inflammatory index*), em adolescentes.

Realizou-se um estudo transversal, com base nos dados do 1º ano do *LabMed and Physical Activity Study*, recolhidos em 2011, numa amostra de 412 adolescentes (idade média de 14.9±1.72 anos), em que a maioria eram raparigas (52.4%). Avaliou-se a inflamação de baixo grau através da medição de bio-marcadores da inflamação, a ingestão dietética e nutricional através de um questionário de frequência alimentar (FFQ – food-frequency questionnaire), a atividade física moderada a vigorosa e tempo de atividade sedentária através da acelerometria e o índice de massa corporal através de medições antropométricas; o desenvolvimento pubertal, o estatuto socioeconómico e os hábitos tabágicos foram auto-relatados.

Os bio-marcadores da inflamação foram medidos em amostras de sangue. As amostras foram colhidas, estando os adolescentes em jejum, refrigeradas e enviadas a um laboratório para determinação dos seguintes: proteína C reativa (CRP – C-reactive protein), interleucina-6 (IL-6), componentes do complemento 3 (C3) e 4 (C4). Foram criadas categorias de baixo ou alto (estado inflamatório) para cada um dos bio-marcadores da inflamação, considerando os valores da

mediana ajustados para o sexo e a idade. Uma pontuação global dos bio-marcadores da inflamação foi definida através da soma dos bio-marcadores da inflamação em categorias, em que se atribuiu um ponto por bio-marcador para os indivíduos que tinham valores acima da mediana ajustada ao sexo e idade, ou zero para os que tinham valores abaixo. A pontuação global dos bio-marcadores da inflamação calculada foi classificada em 0–1 ou 2–4 bio-marcadores altos ou acima da mediana.

A ingestão alimentar e nutricional foi estimada através do FFQ e do *software* de análise nutricional *Food Processor Plus*. As variáveis relacionadas com a ingestão alimentar e nutricional foram definidas de forma diferente em cada manuscrito. No artigo I, o consumo de ácidos gordos foi calculado em percentagem do valor de ingestão energética total (%en) e categorizado de acordo com os respectivos tercis. No artigo II, os consumos de fruta, hortícolas e sopa de hortícolas foram avaliados em porções diárias e categorizados em <3 ou ≥3 porções/dia para as frutas e para os hortícolas, bem como em <2 ou ≥2 porções/dia para a sopa de hortícolas. Avaliou-se a adesão à recomendação "*5-a-day*" (mensagem de saúde pública para incentivar o consumo de pelo menos 5 porções diárias de produtos hortofrutícolas conhecida em Portugal por "5 ao dia") através da soma do número de porções diárias consumidas de fruta, hortícolas e sopa, e seguidamente criadas categorias de <1, 1–5 ou ≥5 porções/dia. No artigo III, a ingestão de uma determinada variedade de frutas e de hortícolas foi calculada considerando o número itens do FFQ de frutas ou hortícolas consumidos (os itens consideram frutas e hortícolas individualmente ou combinados) "pelo menos uma vez por mês" em categorias/mês, e de seguida categorizada de acordo com os respectivos tercis. No artigo IV, a pontuação do DII foi calculada com base em 31 parâmetros alimentares ou nutricionais e categorizada de acordo com os respectivos tercis.

Os *odds ratios* (OR), os intervalos de confiança a 95% (IC95%) e os valores de p para a tendência (p_{trend}) foram calculados através de regressão logística binária, ajustada para variáveis confundidoras, para estimar a magnitude da associação entre os bio-marcadores da inflamação e a pontuação global dos bio-marcadores da inflamação, como variáveis dependentes, e a ingestão alimentar e nutricional,

como variáveis independentes. As variáveis confundidoras consideradas foram sexo, idade, atividade física moderada a vigorosa, tempo de atividade sedentária, índice de massa corporal, desenvolvimento pubertal, estatuto socioeconómico e hábitos tabágicos, para todos os artigos, bem como ainda o consumo total de energia, para os artigos II ao IV.

No artigo I, observou-se que os adolescentes com um maior consumo de ácidos gordos saturados (≥11,1 %en), quando comparado com um menor consumo (≤9,7 %en), tiveram maior probabilidade de ter maior nível de IL-6 (OR=2,16; IC95%=1,02-4,56; p_{trend}=0,051), e maior pontuação global dos bio-marcadores da inflamação (OR=2,51; IC95%=1,20-5,27; p_{trend}=0,051). Além disso, os adolescentes que apresentaram um elevado consumo do ácido α-linolénico (ALA) (≥0,50 %en) e dos ácidos eicosapentaenóico e docosahexaenóico (EPA+DHA) (≥0,22 %en), quando comparados com aqueles que tiveram um consumo mais baixo (≤0,42 %en e ≤0,12 %en, respetivamente), tiveram uma menor probabilidade de ter uma pontuação global dos bio-marcadores mais elevada (OR=0,49; IC95%=0,26-0,95; p_{trend}=0,047 e OR=0,46; IC95%=0,23-0,93; p_{trend}=0,025, respetivamente).

No artigo II, mostrou-se que os adolescentes com um consumo de sopa de hortícolas <2 porções/dia tiveram uma maior probabilidade de ter um elevado nível de IL-6 (OR=12,95; IC95%=1,63-102,84; p<0,05) quando comparado com um consumo ≥2 porções/dia. Além disso, os adolescentes que apresentaram uma ingestão de frutas e hortícolas <1 porção/dia, quando comparada com ≥5 porções/dia (adesão à recomendação *5-a-day*), tiveram uma maior probabilidade de ter um elevado nível de CRP (OR=5,24; IC95%=1,62-16,97; p_{trend}=0,005), IL-6 (OR=5,36; IC95%=1,64-17,53; p_{trend}=0,008), C4 (OR=3,39; IC95%=1,12-10,28; p_{trend}=0,016), e uma maior pontuação global dos bio-marcadores da inflamação (OR=8,14; IC95%=2,44-27,18; p_{trend}<0,001).

No artigo III, verificou-se que os adolescentes que tiveram uma maior variedade no consumo de produtos hortícolas (≥13 categorias/mês) apresentaram uma menor probabilidade de ter um elevado nível de CRP (OR=0,31; IC95%=0,15-0,64; p_{trend}=0,004), quando comparados com os adolescentes com uma menor

variedade no consumo (≤6 categorias/mês), independente da quantidade de hortícolas consumida.

No artigo IV, encontrou-se que os adolescentes classificados no terceiro tercil para a pontuação do DII (maior intensidade inflamatória da alimentação), quando comparados com o primeiro tercil (menor intensidade inflamatória da alimentação), tiveram uma maior probabilidade de terem um elevado nível de IL-6 (para todos os modelos: modelo não ajustado, OR=1,88; IC95%=1,09-3,24; p_{trend}=0,011; depois do ajuste para o sexo, OR=1,84; IC95%=1,06-3,18; p_{trend}=0,015; bem como para o modelo ajustado para todos confundidores, OR=3,38; IC95%=1,24-9,20; p_{trend}=0,023). Ainda, os adolescentes que apresentaram uma pontuação mais elevada do DII (terceiro tercil) tiveram uma maior probabilidade de ter níveis elevados de C4 (0R=3,12; IC95%=1,21-8,10; p_{trend}=0,016) e uma maior pontuação global dos bio-marcadores da inflamação (OR=5,61; IC95%=2,00-15,78, p_{trend}=0,002), nos modelos ajustados para todos os confundidores.

Em suma, a inflamação de baixo grau foi associada positivamente com a ingestão de ácidos gordos saturados e negativamente com a ingestão de ALA, de EPA+DHA, de sopa de hortícola, de maior variedade de hortícolas, e com um padrão alimentar considerado pró-inflamatório.

Os resultados desta tese suportam as recomendações para os adolescentes de limitar o consumo de ácidos gordos saturados e promover o consumo de ácidos gordos polinsaturados da série n-3, especialmente o ALA, o EPA e o DHA, bem como diariamente consumir duas porções de sopa de hortícolas, aderir à recomendação *5-a-day*, e consumir uma variedade de hortícolas. Além disso, o DII pode ser uma ferramenta útil para avaliar o potencial inflamatório da alimentação dos adolescentes, bem como para avaliar a sua associação com bio-marcadores da inflamação.

3. List of abbreviations

AA	Araquidonic acid
AHEI	Alternative Healthy Eating Index
ALA	Alpha-linolenic acid
CRP	C-reactive protein
C3	Complement component 3
C4	Complement component 4
DALYs	Disability-adjusted life-years
DASH	Dietary Approaches to Stop Hypertension
DHA	Docosahexaenoic acid
DII	Dietary inflammatory index
EDII	Empirical dietary inflammatory index
EPA	Eicosapentaenoic acid
FFQ	Food frequency questionnaire
HEI	Healthy Eating Index
HOMA-IR	Insulin resistance homeostatic model assessment index
IL-6	Interleukin 6
LA	linoneic acid
n-3	Omega 3 fatty acids
n-6	Omega 6 fatty acids
MUFA	Monounsaturated fatty acid
OR	Odds ratio
p_{trend}	p values for trend
PUFA	Polyunsaturated fatty acid
SFA	Saturated fatty acid
TFA	Trans fatty acid
TNF-α	Tumor necrosis factor alpha

WHO	World Health Organization
95%CI	95% confidence intervals
%en	percentage of energy intake

4. Background

4.1. Nutrition in Adolescence

Adolescence is a transition phase from childhood to adulthood; it is a period of life cycle when great brain maturation and physical growth occur, and it is characterized by physical, psychological, and emotional changes.[1] Although most adolescents enjoy better health than most other age groups,[2, 3] all of these changes have a direct effect on the nutrients needs and food consumption.[4] The nutritional and dietary intake of adolescents is not only determinant of adolescent growth and development, but they also predict nutrition and health in later life, with high implications for public health.[3] Dietary habits and preferences developed during childhood may be maintained into adulthood.[5] Equally, food intake in adolescence is a predictor of intake in adulthood.[6] So, adolescents have the last opportunity to acquire healthy eating habits that will last into adulthood.

The following topics will be described: a brief overview of the growth and development processes that occurs during adolescence, considering some important factors for adolescent health; the nutritional and dietary needs of adolescents for achieving optimal growth and development; and finally, the eating habits of adolescents, with a special focus on Portuguese individuals to understand whether they comply with the nutritional and dietary recommendations.

4.1.1. Growth, development, and health

The World Health Organization (WHO) defines adolescence as a period of life when people are between 10 and 19 years of age. However, other terms like child, youth, and young people can have definitions that include all or some adolescents. The Convention on the Rights of the Child (1969) defined a child as someone under the age of 18 years; youth refers to those who are between 15 and 24 years old; and young people includes both adolescents and youths together, that is, people between 10 and 24 years old.[1-3] Yet, adolescence can be divided into early (10–13 years), middle (14–16 years) and late (17–19 years) adolescence;[3] or simply early (10–14 years) and late (15–19 years) adolescence.[2]

4.1.1.1. Health issues

Adolescence is considered the period of life where people enjoy better health, and have low mortality rate in most of countries.[2] In 2013, the leading causes of death were road injuries, both among adolescents around the world and in Portugal.[7] The mortality rate in Portuguese adolescents is one of the lowest among all age groups, being just greater than children between 1 to 9 years (Figure 1).[8] So why study adolescents in the context of health?

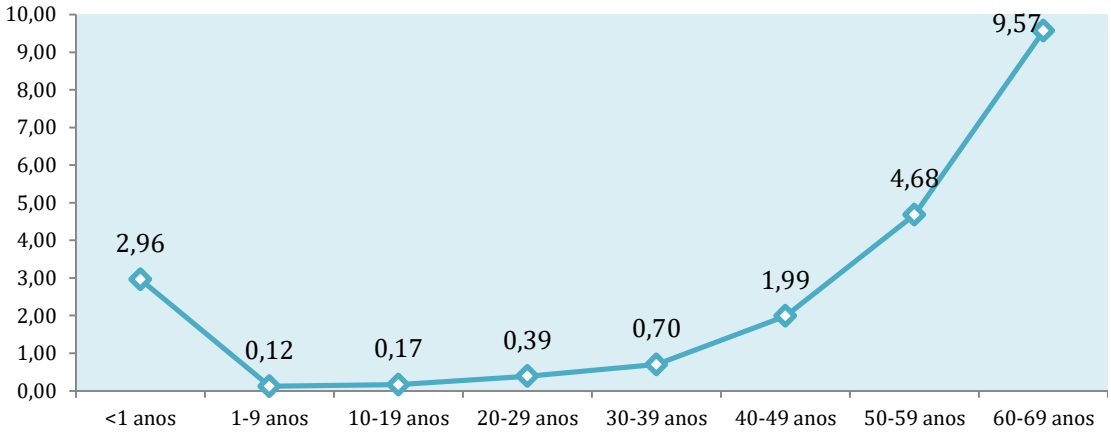


Figure 1: Mortality rate per thousand by age group, Portugal, 2013-2015
Source: Graphic developed based in data from *Instituto Nacional de Estatística*, Portugal.[8]

A single mortality rate may be underestimating the potential importance of the contribution of adolescent health to overall public health. For example, the prevention of an initiation of a smoking habit, which typically occurs during adolescence, could substantially reduce the burden on future health and health systems.[9]

In addition, during adolescence, individuals have best opportunity to improve their own health capital, which normally reaches its peak at this stage of life. Health capital is a kind of health resource and is determinant of health throughout the lifecycle. For example, physical fitness and bone mineral density reach their peak around the age of 20 and they are predictive of cardiovascular disease and osteoporosis, respectively, in later life.[2] So, a health pattern of diet and physical activity during adolescence remains very important to improving health capital and preventing chronic diseases. Furthermore, the lifestyles of adolescents, including diet, physical activity, and overweight, may significantly

impact on causes and prevention of a state of low-grade inflammation, that play a role in the promotion and progression of chronic disease.[10]

It is also important to recognize that the adolescents of today are the parents of tomorrow, and their health capital will be very determinant of the health for their children, in early life.[2] The health of offspring is dependent on maternal health like obesity and on health-related behaviors like alcohol consumption, and they will affect the health of their babies.[11] Early life origins of low-grade inflammation is already recognized, once babies with lower birth weight have higher probability of have low-grade inflammation during childhood and adolescence.[12] Also, impaired fetal growth, of which the risk is increased in adolescent pregnancy, is a potent precursor of adult diabetes,[11] also very closely related with low grade inflammation.[13, 14] So, optimizing pre-natal growth becomes a key factor in human capital formation,[15] and early healthy eating patterns play a important role. Adequate nutrition during adolescence should be provided to supply all the nutrients necessary to achieve full growth and positively impact adult mortality and morbidity,[15] including the prevention of disease closely related with low-grade inflammation, such as obesity, diabetes, cardiovascular disease, and stroke.[13, 14, 16-18]

Also, adolescents have an opportunity to rectify problems that started in childhood.[3] For example, overweight and obesity among children is a health problem recognized all over the world and closely connected with low-grade inflammation.[19-21] Although some countries showed that the prevalence of obesity may have stabilized[22] or slightly declined,[23] it still remains high.[22, 24] Among 7-year-old children in Portugal, the prevalence of overweight and obesity (according WHO cutoff points) is estimated to be 23.8% and 16.7% for boys, and 22.9% and 12.6% for girls, respectively.[25] In addition, when obesity starts in childhood, the probability of becoming obese in adulthood is higher,[26-28] along with an increased risk of being an adult with chronic diseases.[29-31]

Finally, being in good health enable adolescents to deal with the challenges of growing.[32] So, adolescent morbidity and the assessment of diseases and conditions that develop during adolescence and have repercussions throughout life also become important.[3] The disability-adjusted life-years (DALYs), the

equivalent of years of full health lost, is a measure of overall burden of disease. As shown in a systematic analysis by Gore et al.,[9] while some health risk factors like alcohol intake at age 10 contributing to DALYs at an early age, other factors like overweight and obesity and other cardiovascular disease risks only begin to produce effects later in life.

Despite a low mortality rate among adolescents, the prevalence of some chronic conditions is considered high: obesity[22, 24, 33] and consequently type 2 diabetes[34, 35] and cardiovascular disease risks.[36, 37] Though no significant changes were observed in the prevalence of overweight and obesity among Portuguese adolescents in the past decade, the prevalence remains high,[33] and was estimated to be between 13.4-28.6% and 3.2-13% for boys and between 8.8-25.6% and 0.6-5.8% for girls (according International Obesity Task cutoffs), respectively.[38]

So, health during adolescence has an impact throughout life and it represents an opportunity to correct problems before they become more serious. Moreover, adolescent health has an essential impact on the health of the next generation. And finally, many adolescents today are overweight or obese, increasing the risk of type 2 diabetes and factors for cardiovascular diseases, with repercussions later in life.

4.1.1.2. Sexual maturity

Though age is a convenient way to define it, adolescence is a broad term that includes puberty, psychological, and social adaptation to the growth events of puberty. In reality, however, the biological changes of adolescence do not all begin at 10 years and stop at 20 years.[3] Indeed, chronological age cannot accurately predict the nutritional needs related to maturation state measures, due to the variability of ages in which the growth spurt may occur.[39]

Importantly, puberty occurs during adolescence, so this period represents the time when adolescents acquire the ability to reproduce.[40] Endocrinal changes (gonadarche and andrenarche) also occur, including hormones that affect gonadal maturation and the production of gonadal sex steroids.[3] This is regulated by the hypothalamo-pituitary-gonadal axis and characterized by the

acquisition of secondary sexual characteristics.[40] Pubertal development can be ranked by the sexual maturity rating or Tanner stages, which identify charts based on secondary sex characteristics (e.g. the development of genitals for boys, breasts for girls, and pubic hair for both).[4, 39] The Tanner stage is assigned on a scale of 1 (prepubertal development) to 5 (complete development – adult).[4, 41]

In addition, spermarche, or the onset of sperm production, occurs at approximately age 14 among boys; while the occurrence of menarche, or the onset of menstruation, occurs at approximately age 12.4 among girls.[42]

4.1.1.3. Physical growth

Pubertal maturation correlates remarkably well with physical growth and hormonal changes.[42] The physical growth include increases in height as well as changes in weight and body composition.[43]

Height: Puberty starts in early adolescence, accompanied by rapid physical growth.[11] The linear growth spurt begins earlier in girls (on average in stage 2) than in boys (on average in Tanner stage 4).[42] During late adolescence, this growth is slowed for girls but continues for boys. At the end of adolescence, girls have their physical development completed, while boys complete themselves in young adulthood (20-24 years).[11] Height increases between 5-25 cm in most girls, and 10-30 cm in most boys. Adequate nutrition is very important in this period; once linear growth is delayed or slowed among adolescents who severely restrict their caloric intake,[42] they may not be able to achieve their full potential height.[43]

Weight: Following linear growth, lateral growth or weight gain occurs. An overlap between height and weight growth is expected,[43] and the weight gain peak follows the linear growth spurt by 3-6 months in girls and by approximately three months in boys.[42] The weight gains vary widely among adolescents, because this is very sensitive to external factors, such as dietary intake and physical activity.[43] However, most healthy boys gain between 7-30 kg during puberty, while most healthy girls gains between 7-25 kg.[42]

Body composition: Together with weight gains, changes in body composition are also observed during adolescence. Most healthy boys decrease their body fat

percentage while increasing lean body mass. Body composition changes more significantly among girls, such that they have a decrease of lean body mass percentage and an increase in body fat levels.[42, 43] Also, a great bone mass is accumulated during adolescence, where more than 90% of adult skeletal mass has been accrued.[42, 44]

Considering those physical changes, the nutritional requirements increase considerably during puberty, and inadequate nutrition leaves the adolescent vulnerable to any deficit, imbalance, or excess, compromising the levels of health and disease prevention that can appear in adulthood.[45] Nutrition, including energy and specific nutrient intake, is considered a major determinant of growth. Under nutrition is the single most important cause of growth retardation worldwide[46] because there is an increase in the nutritional needs during this adolescent growth spurt.[47] On the other hand, caloric excess may favor a positive energy balance and excessive weight gain.[48] A nutrition imbalance, such high saturated fat and low unsaturated fat, can lead to dyslipidemia;[48] and inadequate micronutrients, like vitamin D and calcium, play a important role in skeletal health and the prevention of adult osteoporosis.[42] So, nutrition is important during this time to ensure optimal growth and help prevent chronic diseases during adolescence and in adulthood.[42]

In addition to nutrition, adolescent physical activity has a valuable role during growth and development, with multiple benefits for physical health.[49] During puberty, physical activity may be especially important for optimal bone growth; active adolescents have greater bone mineral content and density than their less active peers. Active adolescents will have reduced osteoporosis-related fracture risk in later life by increasing bone mineral accrual during development; by enhancing bone strength; and by reducing the risk of falls as a result of improved muscle strength, flexibility, coordination, and balance.[49]

Moderate physical activity promotes favorable changes in body composition.[46] Although regular physical activity has no established effect on linear growth rate or ultimate height,[49] it is inversely related to weight and body fat, and positively related with fat-free mass. Physical exercise can induce muscle hypertrophy and strength, and decrease the adipocyte hyperplasia by limiting hypertrophy. It is

known that the increases in the number of adipocytes during puberty are considered critical for enlargement of the adipose tissue organ and for the risk of obesity. Higher amounts of visceral adipose tissue are associated with greater risk of metabolic complications,[49] such as metabolic syndrome and cardiovascular disease, and also associated with increased low-grade inflammation in youth.[20, 50, 51]

Physical activity is central to health, and its importance extends beyond its role in achieving energy balance to prevent and treat obesity and overweight. Adequate daily physical activity improves cardiovascular health; metabolic health; brain and mental health; and musculoskeletal health, benefits that recent research shows are gained across the life span.[49] Regular physical activity contributes to the primary and secondary prevention of several chronic diseases and is associated with a reduced risk of premature death.[52] Adolescents that experienced appropriate physical activity program will be inspired to engage in physical activity for a lifetime. So, physical activity has both immediate and long-term health benefits, because it reduces the morbidity risk in adolescence and can influences future morbidity.[49] Providing opportunities for adolescents to be physically active is important to ensuring adequate growth and development, and good health.

4.1.1.4. Psychosocial changes

In addition to the physical changes, adolescence is also characterized by psychological, cognitive, and emotional changes.[1] Adolescents develop a stronger recognition of their own personal identity; moral and ethical values; and a deeper perception of their feelings. The increased need for energy and nutrients among adolescents, combined with increased financial independence, increased need for autonomy when making food choices, and immature cognitive abilities, can place adolescents at nutritional risk.[42]

During early adolescence, individuals become increasingly influenced by peer group and attracted to testing rules and limits, while during the late adolescence, they tend to distance themselves from theirs parents and have a heightened capacity for emotional regulation.[11] The peer influence is a dominant psychosocial issue during adolescence, especially in the early period, and the

desire to conform can influence food intake. Some individuals can tend to divide food into two groups: junk food and healthy food. Eating junk food is associated with being with friends, having fun, gaining weight, and guilt; whereas eating healthy foods is associated with family, family meals, and home life. Food choices can frequently be based on associations with feelings of being accepted and having fun with peers and adolescents may use food as a way to exert independence from families and parents.[42]

Another worrying psychosocial issue is the trend to break rules and engage in impulsive behaviors. This trend, together with the increased importance of peer acceptance, may influence adolescents to initiate health-compromising behaviors such as smoking, consuming alcohol, using drugs, and engaging in sexual activities, especially during middle adolescence. Later, older adolescents with a strong personal identity are able to suppress impulsive behaviors, and are less affected by peer pressure and more by relationships with single individuals. In this stage, adolescents develop an ability to comprehend how current health behaviors affect long-term health status.[11]

During adolescent growth and development, the social environment can influence health, and social disadvantages in adolescence are partly a determinant of health inequalities in adult life. Social inequalities in health are traditionally measured by examining differences in socioeconomic status.[32] An adolescent's socioeconomic status can be assessed with the family affluence scale,[53] which was developed specifically to measure socioeconomic status in the context of the Health Behavior in School-Aged Children Study, ranking adolescents by their socio-economic status.

Adolescents with high family affluence are more likely to have a higher self-rated health, life satisfaction, and lower probability of being overweight; they have better health behaviors like higher fruit intake, lower soft drink intake, increased likelihood of eating breakfast daily, higher moderate-to-vigorous physical activity, and low prevalence of watching two or more hours of television every weekday; they also have lower risk behaviors, like the onset of a smoking habit.[32] In addition, social disadvantage, specifically low parent education, is associated with increased inflammation in adolescence.[54-56]

So, adolescence is a critical period in determining adult health related behavior in which food habits, physical activity, and social environment are all crucial to health throughout life.

4.1.2. Nutritional needs and dietary recommendations

The nutritional needs of adolescents have been extensively reviewed in several publications.[57-63] A brief overview of some of the nutritional needs and dietary recommendations often highlighted in the literature will be described.

Before adolescence, nutritional needs are similar for boys and girls. During puberty, however, sex-specific nutrient needs develop based on body composition differentiation and biological changes. The nutritional needs for both boys and girls increase in this period, parallel with the rate of growth and with the greatest demands during the growth spurt.[42]

The United State dietary reference intake developed by the Food and Nutrition Board[57] describes the nutritional needs by sex and age range,[58, 59] including approximately early and late adolescence. However, this division by age should be interpreted cautiously when analyzing the reported nutritional requirements by dietary reference intake for adolescents,[47] since chronological age is not the same of pubertal age, as already described. So, pubertal maturation and anabolic state should also be considered to assess adolescent nutritional needs, being the nutritional needs higher during periods of intensive growth, such as the growth spurt.[42]

Energy: The need for energy during adolescence is the average dietary energy intake predicted to maintain an energy balance in a healthy individual of a defined age, gender, height, weight, and level of physical activity consistent with good health, and includes the needs associated with the deposition of tissues, according to the Food and Nutrition Board.[59] The basal metabolism is associated with lean body mass, so boys have higher energy requirements than girls, since they experience greater increases in height, weight, and lean body mass.[42, 44] Some authors recommend the use of height, rather than weight, to calculate caloric requirements, due to the wide variability in the timing of growth and maturation among adolescents,[42] but it is difficult to be implemented.

Estimation of energy requirements are made considering a reference weight and height, depending on age and physical activity level, leading to the following range of values: 1.445-2.585 kcal/day for girls, and 1.576-3.804 kcal/day for boys.[59]

Carbohydrates and dietary fiber: The primary role of carbohydrates is to offer energy, and a range of 45-65% of total daily calories is recommended, limited to a maximal intake of no more than 25% of total energy from added sugars, according to Food and Nutrition Board.[58, 59] However, according to the WHO, added sugars should be limited to no more than 10% of calories per day, with a further reduction to below 5% to prevent body weight gain.[64] These different considerations of different carbohydrates may impact the glycemic index and glycemic load, which measure the glycemic potential of food.[59] High glycemic index and glycemic load have been proposed to be associated with increased risk of chronic diseases,[65] and seem to have a role in low-grade inflammation.[66] There are no specific recommendations for ideal values of glycemic index and glycemic load developed for healthy people, due to the lack of epidemiological evidence linking glycemic index and glycemic load to heart disease, insulin sensitivity, type 2 diabetes, dyslipidemia, and obesity among healthy people.[65] Values of glycemic index and glycemic load are heavily influenced by extrinsic and intrinsic food related factors, such as dietary fiber, which is defined as non-digestible carbohydrates and lignin that are intrinsic and intact in plants, and plays a role in the prevention of several chronic conditions. Colon biota can digest some of dietary fiber and the energy yield of fibers in humans is still unclear, but the range is likely between 1.5 to 2.5 kcal/g. The dietary recommendation of dietary fiber is 14 g per 1.000 kcal, according to Food and Nutrition Board, based on the intake level observed to protect against coronary heart disease. So, adolescent girls should consume on average 26 g/day, while boys in early adolescence should consume 31 g/day and boys in late adolescence should consume 38 g/day.[59, 67]

Fat and fatty acids: Fats represent another important source of energy. Dietary fat and essential fatty acids are necessary for the normal growth and development of adolescents,[42] and intake between 25-35% of calories should

be considered, according to the Food and Nutrition Board.[58, 59] The quality of fat consumption is equally important, since dietary fatty acid intake can impact the development of common diseases, such as cardiovascular disease[59, 68] and diabetes,[68] by the modulation of known and emerging risk factors, such as chronic low-grade inflammation.[69, 70] So the intake of trans fatty acids (TFA) and saturated fatty acids (SFA) should be as low as possible, according to the Food and Nutrition Board,[58, 59] and no more than 1% and 7-10% of calories per day, respectively, according to other organizations.[68] An intake of omega-6 (n-6) and omega-3 (n-3) polyunsaturated fatty acids (PUFA) between 5-10% and 0.6-1.2% of energy, respectively, is considered appropriate according to the Food and Nutrition Board.[58, 59] For n-6 PUFA is considered specially linoleic acid (LA), while for n-3 PUFA is considered especially α-linolenic acid (ALA). However, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), both n-3 PUFA, may provide beneficial health effects, greater than other n-3 PUFA, so approximately 0.06-0.12% of energy (according to the Food and Nutrition Board)[58, 59] or 250-500 mg/day of EPA and/or DHA (according to other organizations)[68] are recommended.

Protein: The need for protein is more correlated with the growth spurt than with age, as is the case for the need for energy.[42, 47] The protein requirements of adolescents are influenced by the amount of protein required for maintenance of existing lean body mass and accrual of additional lean body mass. Reductions in linear growth, delays in sexual maturation, and reduced accumulation of lean body mass may be observed if protein intakes are consistently low.[42] It is unlikely, however, that adolescents in Europe and the United States are at risk of energy or protein deficiency.[42, 47] The dietary recommendation of protein is 0.95 g/kg of body weight per day in early adolescence, and 0.85 g/kg of body weight per day in late adolescence.

Minerals and Vitamins: As energy and protein needs increase, adolescents also exhibit higher vitamin and mineral requirements than people in other periods of life.

Minerals like calcium, iron, and zinc are particularly important for adolescents. Calcium plays a key role in several domains of health including bone health[60]

and the accelerated developments of skeletal, muscular, and endocrine systems at this stage of life make calcium critical in this period.[47] An adequate intake of calcium is essential to developing dense bone mass and reducing the risk of fractures and osteoporosis in early adulthood.[42] The dietary recommendation is 1300 mg/day.[60]

Iron is a critical component of several proteins, including myoglobin and hemoglobin, that transport oxygen throughout the body.[61] Iron requirements increase during puberty due to expanding blood volume for both sexes, but also to increase muscle mass in boys and replace blood loss due the onset of menstruation in girls.[42, 47] The recommended daily requirements are 8 mg/day in early adolescence, and, in late adolescence, 11 mg/day for boys and 15 mg/day for girls.[61] Iron can exist in various oxidation states, including the ferrous, and ferric states,[61] having a redox potential.[71] It has a complex relationship with inflammation responses, being called a double-edged sword, as both iron deficiency and excess can have deleterious effects.[69]

Zinc is crucial for growth and development[61] and serum zinc levels experience a decrease in response to the rapid growth and hormonal changes.[42, 47] Zinc requirements are 8 mg/day in early adolescence, while in late adolescence, girls require 9 mg/day and boys require 11 mg/day.[61] It deficiency, especially in boys, can leads with growth failure and delayed sexual development.[42, 47] Zinc deficiency may also increase cell-mediated immune dysfunctions, oxidative stress, and inflammatory cytokines.[72]

Vitamins are vital for good growth and development of adolescents, who often have an increased need in this period. Folate has an integral role in DNA, RNA, and protein synthesis, so it is increased during puberty. The dietary recommendation of folate is 400 μg/day for adolescents[63] and adequate intake is especially important for girls who may become pregnant to reduce the incidence of congenital anomalies among offspring.[42]

Vitamin D maintains serum calcium and phosphorus concentrations, so it is involved in bone health, making it very important during adolescence due to the skeletal mass increase.[60] A pro-inflammatory role of vitamin D has also been suggested, but it has several anti-inflammatory actions, such as inhibiting the

proliferation of lymphocytes, inducing their apoptosis, and regulating the expression of cytokines and adhesion molecules.[69] Vitamin D can be obtained by exposure to sunlight, and the recommendation of 5 μg/day during adolescence is based on the absence of adequate exposure to sunlight.[60]

Vitamin A is also considered a nutrient of special concern during adolescence, playing a vital role in reproduction, growth, and immune function.[61, 73] Dietary recommendations for vitamin A are 600 μg/day in early adolescence, and in late adolescence, 700 μg/day for girls and 900 μg/day for boys. Vitamin A deficiency is associated with increased susceptibility to infectious diseases, and vitamin A also has a role as an anti-inflammatory agent.[74]

Vitamin E has a function in cell membranes and so becomes increasingly important during adolescence due expansions of body mass during this period,[42] but its major function appears to be its role as the most effective chain-breaking antioxidant. Dietary recommendations for vitamin E are 11 mg/day in early adolescence and 15 mg/day in late adolescence.[62]

Vitamin C is involved in the synthesis of collagen, is important for growth,[42] and plays a protective antioxidant role. Along with vitamin E, vitamin C is considered a dietary antioxidant and may play a role in the prevention of chronic diseases,[62] as well as in decreasing inflammation.[69]

Dietary recommendations: Contrary to what happens to energy and protein, in which deficiency is unlikely, the eating habits of adolescents may put them at risk of deficiency for vitamins and minerals.[42] The adequate intake of some food categories like fruits, vegetables, and dairy products, while also avoiding soft drinks and smoking may help to prevent deficiencies, especially for some vitamins and minerals.[47] Adolescent vegetarians may be at the highest risk for low mineral intake, especially minerals present in animal-based foods, and supplementation may be warranted.[42]

To avoid nutritional inadequacy and meet their daily nutrient needs, most people should consult the dietary guidelines to guide their food choices. The new food guide for the Portuguese indicates a range of daily recommended portions, by food categories, allowing individuals to maintain a healthy diet. Adolescents,

primarily boys, should consume the highest number of portions because they have higher energy needs.[75] For example, between two and three portions of dairy products are recommended every day, while adolescent should eat three portions for good bone health.[76]

Three to five daily portions of fruits and vegetables are recommended.[75] Following this recommendations is especially important in the promotion of health and the prevention of many chronic diseases.[77] The WHO recommends at least 400 g daily of these foods categories,[78] with an increase up to 600 g to reduce the global burden of cancer and cardiovascular diseases.[79] The '5-a-day' recommendation may be a simple, easy-to-remember guide to help individuals to follow a minimal daily intake of any combination of fruits and vegetables.[80] In addition to choosing the right number of portions, the WHO[81] and the United States[82] dietary guidelines also indicate a need to choose a wide variety of these foods. For this last report (United States dietary guidelines[82]), a separation of fruits and vegetables is observed, with a recommendation to eat a variety of vegetables, beyond the general recommendation of to eat a variety of nutrient-dense foods.

4.1.3. Nutritional and dietary intakes

Energy intake increases with age, and this is very similar between the sexes, in early adolescence. This increase continues until age 18 for boys, but decreases in middle or later adolescence for girls. Possible under-reporting of food habits or dietary restrictions in this period may occur between them.[83]

Among Portuguese adolescents, starchy foods are the first contributor to energy intake.[84] Among Europeans, the absolute amount (g) of total carbohydrates and fiber is higher for boys,[83] in accordance with recommendations. The southern Europeans, including Portuguese, have a percentage of energy from starch very similar to trend of energy intake, increasing with age; in contrast, sugars tended to decrease with age.[83] Most adolescents have their total carbohydrates intake within recommended levels, between 52-53.9%,[85] but close to the lower end of this range; on the other hand, their consumptions from added sugars may

provide more than 10% of the energy intake, which is higher than the daily recommendation.[85]

Adolescents in Southern European countries tend to have a higher intake of total fat and monounsaturated fatty acids (MUFA) than those in Northern, Central, and Eastern European countries.[83] Among Portuguese, the fat intake is about 31 to 33% of the total energy intake,[85, 86] which is closer to the upper end of the recommendations. The consumption of olive oil, which is higher among Mediterranean countries, may explain the highest intake of MUFA.[85] However, meat consumption is the largest contributor to the intake of total fat and MUFA among Portuguese adolescents.[84]

European adolescents 10 and 11 years old are rarely consumers of alcohol. However, from the age of 11 years, a clear trend of initiation and increasing alcohol intake is observed more among boys than girls. Typically, young adolescents (11 years) have an alcohol intake of about 1.5 g/day (0.5% of energy), which increases to 10 g/day (3.3% of energy) for boys and 6 g/day (1.8% of energy) for girls, when they reach 15 to 18 years old.[83] Among 15 and 16 year old Portuguese adolescents, about 52% consumed alcohol (i.e. any alcohol intake over the past 30 days) in 2011.[87] Although, a trend towards decreasing weekly alcohol consumption in European and North Americans countries has been observed between 2002 and 2010, Portugal has shown no such trend.[88]

In general, European adolescents increase their intake of micronutrients with age, in parallel with energy intake.[83] However, there is a risk of deficiency of calcium, iron, and zinc among Portuguese, mainly girls.[85] Dairy products are the main source of calcium in the diet of Portuguese adolescents,[84] and it seems that the minimum needs are fulfilled for both dairy products and calcium, since the average intake is, respectively, 2.3 or 2.1 portions/day and 1311 or 1215.2 mg/day (boys or girls), in a sample of Azorean 15-18 year olds.[89]

Considering others food categories, the mean of fruit and vegetable consumption among Europeans adolescents is bellow the recommendation, varying from 220 to 345 g/day, and only 23.5% of adolescents reach the WHO goal of 400 g/day.[90-93] Among Portuguese, the intake was assessed as 137 g/day for fruit

and 112 g/day for vegetables, with only 21% of them reaching the WHO goal[91] of 400 g/day of both fruits and vegetables.[78] In addition, no differences were found between sexes.[91] The vegetable soup may give a valuable contribution to the daily intake of vegetables, and it was estimated on average 0.8–0.9 times a day among adolescents.[94] An increase in the daily fruit and vegetable consumption was noted among adolescents from most European and North American countries, from 2002 to 2010. Unfortunately, the prevalence of daily fruit consumption in Portugal has significantly decreased. For vegetables, no change was observed.[95]

Daily breakfast consumption across 41 countries, including more them 200 000 adolescents,[96] is common skipped among adolescents, especially girls, older adolescents and those from social disadvantaged. Daily breakfast consumption was associated with life-styles, such as being physically active and daily fruit or vegetable consumption that were positively associated, as well as watching >2 hours/day of television, soft drink consumption and dieting was negatively associated. Portuguese adolescents were among those that have higher daily breakfast consumption. However, 30% of girls and 25% of boys have reported to skipped breakfast.

Soft drink consumption among Americans adolescents is a major source of added sugar in the their diets, accounting for over 12% of all carbohydrates consumed.[42] For Portuguese adolescents, soft drinks account for about 4% of total consumption of energy and about 7% of total consumption of carbohydrates. Soft drinks are the fourth source of carbohydrates, after starchy food, fruits, and sweets/pastries.[84]

Fast food intake among 72 900 children (from 17 countries) and 199 135 adolescents (from 36 countries)[97] was studied and considered high in childhood and increases in adolescence. In addition, consumption of fast food was associated with higher body mass index in both children and adolescent. Among Portuguese children (mean age of 7.5 years),[98] fast food and pastry pattern was positively associated to TV viewing (>2 h/day), while higher level maternal education and longer sleeping duration were positively associated with

dietary patterns that included vegetables, pulses, fruit, olive oil, and vegetable soup, and negatively associated with fast-food and pastry intake.

From a perspective of the complexity of diet, four different dietary patterns were identified among Portuguese adolescents: healthier in 16.1% of adolescents, dairy products in 29.7%, fast food in 14.2%, and lower intake in 40%. In the healthier or dairy products patterns, adolescents had a food intake closer to a healthy diet. On the other hand, adolescents with the fast food pattern had a higher consumption of fast food, sweets, and soft drinks. Adolescents with that food pattern spent more time in sedentary activities and had a lower socioeconomic status.[86]

In addition, time spent watching television was associated with higher consumption of foods rich in fats and sugars and a lower consumption of fruits and vegetables. Adolescents who watched television more than 120 minutes/day had a higher intake of dietary fat and a lower intake of minerals and vitamins.[99]

4.2. Low-grade inflammation in adolescents

Acute inflammation is a normal defense mechanism. If physiological processes to resolve inflammation fail, acute inflammation can become chronic inflammation. By contrast, in low-grade inflammation, there is an increase in systemic inflammatory biomarkers, but their presence is not high enough to characterize low-grade inflammation as acute or typical chronic inflammation. Low-grade inflammation has been associated with risk factors for metabolic and chronic diseases[69, 70, 100] in adults,[13, 14, 16, 18, 101, 102] children, and adolescents.[19-21, 50, 51] Several factors have been shown to influence concentrations of inflammatory biomarkers including body fatness, physical activity, and diet.[69]

In the following sections, four areas will be addressed: a brief overview of the inflammatory process; a distinction between acute, chronic, and low-grade inflammation; a brief review of the most relevant inflammatory biomarkers and their associations with chronic conditions and risk factors in adolescents; and finally, the determinants of low-grade inflammation in adolescents, with a special focus on dietary and nutritional intake.

4.2.1. The inflammatory process

Inflammation is a normal defense mechanism and an essential component of immunity, and it acts as a defense for the host.[70] It is an adaptive response that is triggered by noxious stimuli and conditions, such as infection and tissue injury.[103]

The classic inflammation process has three phases: the afferent, the efferent, and the resolution. The afferent phase occurs when certain types of cells sense the presence of a trigger; the efferent phase is the inflammatory response to eliminate the trigger; the resolution phase is initiated in order to limit further damage to the host and to repair the tissue. The efferent phase involves four major events: 1) first, in the presence of a trigger in the tissue, the blood supply to the site of inflammation is increased; 2) next, capillary permeability is increased due to the retraction of endothelial cells, thereby permitting larger molecules to traverse the endothelium to deliver mediators to the site of inflammation; 3) then, chemo-attractants are released from the inflammation

site and by the up-regulation of adhesion molecules on the endothelium in order to promote the migration of leukocytes from the capillaries into the tissue; 4) in the tissue, the leukocytes move to the site of inflammation where they release mediators such as cytokines.[100] Calder et al.[100] summarized these events and showed that some inflammatory mediators can escape from the inflammation site into the bloodstream and exert systemic effects. This initial inflammatory process leads to the production of a variety of inflammatory mediators, including chemokines, cytokines, vasoactive amines, eicosanoids, and products of proteolytic cascades.[103]

The four events of the inflammatory response process aim to eliminate the perceived hostile intruder, i.e., the source of the trigger. Once the insult is eliminated or at least controlled, mechanisms come into play to terminate inflammation in order to limit further damage to the host and to initiate tissue repair. This process is called the resolution of inflammation, and it is now recognized as an active process involving specific mediators that act to down-regulate the processes that were activated earlier. The resolution phase involves activations of negative feedback mechanisms in which the anti-inflammatory cytokine is secreted to inhibit the pro-inflammatory cascades.[100]

4.2.2. Low-grade inflammation

Inflammation may be classified as acute or chronic. Acute inflammation is the body's initial response to an infectious agent or other inflammatory trigger (e.g., tissue damage caused by a wound or irradiation) and is achieved by the increased movement of plasma and leukocytes (especially granulocytes) from the blood to the site of the infection or injury.[100]

Typically, the inflammatory response is activated rapidly in response to infection or another trigger, and then follows a temporal pattern of cellular activation and chemical mediator release. Once the infection or the other insult has been eliminated or controlled, mechanisms are activated in order to terminate inflammation.[100]

When the process to resolve inflammation fails, the normally acute inflammatory response can become chronic.[69] Chronic inflammation may become

pathological and cause irreparable damage to the host's tissues, resulting in markedly elevated concentrations of inflammatory biomarkers and activated inflammatory cells at the site of tissue damage and in the circulation systemic. This state of inflammation may be regarded 'high grade' and is characteristic of autoimmune, allergic, and neurodegenerative diseases such as chronic diseases, allergic asthma, and Alzheimer's disease.[100]

Chronic inflammation can also be considered 'low grade' when clinical manifestations are minimal or absent. Thus, in the case of low-grade inflammation, the increase in systemic inflammatory biomarkers is not as high as in typical chronic inflammation, and it has been associated with metabolic diseases,[70, 100] such as obesity[13] and diabetes,[13, 14] and with cardiovascular diseases[16, 18] and cancer.[101, 102] This condition of low-grade inflammation is commonly found in the elderly because inflammatory biomarkers tend to increase with age.[104, 105]

However, some of these associations have also been found in children and adolescents, where low-grade inflammation has been associated with several risk factors for chronic conditions including obesity,[19-21, 106, 107] metabolic syndrome,[20, 50] and early-stage atherosclerosis,[51] as summarized in Table 1.

4.2.3. Inflammatory biomarkers

Low-grade inflammation is characterized by raised blood concentrations of inflammatory biomarkers.[69] The early identification of those biomarkers that have diagnostic value for the prevention of future damage or can facilitate early treatment is desirable;[108] however, inflammatory biomarkers are considered non-specific for healthy people, and a definition of biomarkers or a set of them that fully describes the low-grade inflammation has not yet been well defined.[69, 70, 100]

Relevant inflammatory biomarkers in the general population, i.e., in non-disease settings, are cells or soluble markers found in the bloodstream. Tissue markers can also be used, but often they are disease specific. Calder et al.[100] explored several inflammatory biomarkers in order to understand their nutritional and dietary role in inflammation. For this thesis, the focus will be on biomarkers of

inflammation considered most relevant in studies based on apparently healthy adolescents, as shown in Table 1.

4.2.3.1. Acute-phase proteins

The acute-phase proteins are synthetized mainly by the liver and induced by cytokines.[100] C-reactive protein (CRP) is considered a highly sensitive acute-phase protein for marking systemic inflammation and to predicting cardiovascular risk, despite its lack of specificity in differentiating inflammatory diseases.[10] Although the production of CRP takes place primarily in the liver, this biomarker can also be produced by inflammatory cells at the inflammation site at lower concentrations less likely to have systemic effects.[109]

Increased levels of CRP are known to be associated with several conditions such as infection and tissue damage, obesity, old age, hypertension, diabetes mellitus, smoking, and other cardiovascular risks.[110] CRP cut-off points were defined to assess the cardiovascular disease risk in adults, but no reference ranges have yet been defined for adolescents.[10]

In children and adolescents, CRP has been associated with obesity, specifically with body mass index,[19-21] peripheral fat mass,[21] and abdominal obesity,[50] as shown in Table 1. This relationship may have an early etiology, since maternal inflammation during pregnancy can predict adiposity in the offspring during childhood. In a prospective prebirth cohort analysis of mother-child pairs, Gaillard et al.[111] showed that higher maternal CRP levels in the second-trimester of pregnancy were positively associated with higher overall and central adiposity in the offspring's childhood (median age 7.7 years).

High CRP has also been associated with metabolic parameters during adolescence (Table 1), including high fasting insulin, high triglycerides, and low high-density lipoprotein cholesterol concentrations in individuals ages 9, 13, and 16 years;[20] and with metabolic syndrome in adolescents ages 12–17 years.[50] In addition, metabolic abnormalities have a greater effect on CRP than weight alone since overweight adolescents (ages 12–19 years) with metabolic syndrome show higher levels of CRP than their overweight peers without metabolic syndrome.[112] Moreover, CRP plays a role in the pathogenesis of early

atherosclerosis, affecting the health of arteries in healthy children and adolescents, mean age 10 years,[51] in obese adolescents, mean age 13.5[113] years, by disturbing endothelial function and promoting intima-media thickening. In fact, Silva et al.[114] demonstrated that obese had higher values of intima-media thickening and concentrations of CRP compared to non-obese in a meta-analysis based on 16 studies among obese and non-obese children and adolescents.

Other acute-phase proteins less well studied than CRP, such as complement component 3 (C3) and complement component 4 (C4), are soluble membrane-bound proteins that intertwine with the complement system and also play a role in inflammatory processes.[115] C3 and C4 are also synthetized by the liver; however, body fatness seems to contribute to circulating concentrations of these serum proteins,[107, 116] even in apparently healthy adolescents. In adult-population studies, C3 has been associated with body fatness and cardiovascular disease risk factors[117] such as body mass index, visceral adipose tissue, insulin levels, insulin resistance homeostatic model assessment index (HOMA-IR), diabetes, hypertension, and dyslipidemia.[116] As shown in Table 1, higher concentrations of C3 in adolescents have been linked to high body fatness including waist circumference,[107] and body fat percentage,[107, 118] independent of being normal weight or overweight, in a sample of adolescents ages 13–18.5 years,[107] and waist circumference in boys and waist-to-height ratio in girls ages 12–18 years.[118]

The inflammatory biomarker C4[117, 119] and the C3/C4 ratio[120] have been associated with increased cardiovascular risk factors in adults. A very high C4[119] and a high C3/C4 ratio[120] increases the hazard of a new cardiovascular event, and high C4 has been positively associated with body fatness measures, blood pressure, serum lipids, [117, 119] and blood glucose levels.[117] The C3 and C4 levels declined after weight reduction.[117, 121] In children and adolescents, as shown in Table 1, some measurements of body fatness were associated with C4;[107] body mass index, waist circumference, waist-to-height ratio, skinfold thickness, and body fat percentage were positively correlated with that biomarker, but the statistical significance of the association was lost if the

sample was divided into normal weight and overweight groups of adolescents ages 13–18.5 years.[107] In addition, C4 predicted measures of obesity and central obesity in 12–18 year old girls.[118] Other interesting findings were that birth weight was inversely related to C3 and C4 in children and adolescents.[12]

4.2.3.2. Cytokines

Cytokines are a class of high molecular-weight polypeptides that deliver cell signals in the context of immunological responses, inflammatory reactions, and other basic biological functions. Interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-α) are the most well-studied cytokines.[122] They are released from vascular smooth muscle cells, endothelial cells, monocytes, and macrophages.[110] Several pro-inflammatory cytokines are also able to regulate the hepatic synthesis of CRP and the hepatic acute-phase response; however, the role of IL-6 in this process is considered primary.[123]

IL-6 can also be produced by adipose tissue, particularly in the adipose visceral tissue but also subcutaneously by both adipocytes and macrophages.[124] A number of different stimuli including other cytokines, infections, and toxins can induce its production. In acute inflammation, two important functions of IL-6 can condition other inflammatory biomarkers: modulating the synthesis of acute-phase proteins (such as CRP) and self-limiting the inflammatory response by suppressing other cytokines like TNF-α. Thus, IL-6 concomitantly regulates pro-inflammatory and anti-inflammatory activities and contributes to both the development and the resolution of the acute inflammatory response. However, it is not understood yet whether elevated levels of IL-6 are aimed at resolving an inflammatory response that is inappropriately long or if a primary dysregulation of IL-6 production is responsible for a chronic pro-inflammatory state.[125]

IL-6 is involved in cardiovascular disease, possibly due to its effects on insulin resistance, pro-coagulant state, dyslipidemia, and endothelial activation.[123] Cohort studies in healthy male adults and in the elderly show that subjects with higher serum values had a higher incidence of cardiac events such as myocardial infarction, ischemic cardiac disease, stroke, and heart failure.[110] In studies based on adolescent samples, an increase in IL-6 level was found in the presence of several cardiovascular risk factors as shown in Table 1, such as impaired

metabolic processes, for example, in the presence of higher HOMA-IR in adolescents ages 12–18 years;[126] higher clustering z-scores of cardiovascular disease risk factors, including measures of glucose tolerance, serum lipids and body fatness;[127] and higher prevalence of metabolic syndrome[128] in adolescents (mean age 13 years).

In healthy adults, the production of IL-6 increases with adiposity, and estimates indicate that as much as a third of total circulating concentrations of IL-6 originate from adipose tissue.[123] It is unclear whether this plays a role as one of the contributing mediators of obesity[129] or it has a homeostatic role in limiting obesity-associated insulin resistance and inflammation.[130] A recent study of biopsies of adipose tissue from normal weight, overweight, and obese adults suggested that obesity leads to the elevated expression of IL-6 in the adipose tissue.[131]

The findings regarding the relationship between IL-6 levels and body fatness measures are conflicting in younger populations, as exhibited in Table 1. Some studies found an inverse relationship; in adolescent ages 12–18 years, those who were obese had higher serum values of IL-6 than normal weight ones,[126] and in children and adolescents ages 6-19 years, those who had higher levels of IL-6 had a higher waist-to-height ratio.[132] However, several studies based on adolescent samples found no relationship between IL-6 levels and body fatness measures such as body mass index.[107, 133-135] Tam et al.[136] considered that the effects of obesity on IL-6 levels vary with age and sex since overweight and obese girls at 15 years of age but not at 8 years of age, showed increased IL-6 compared to healthy-weight girls in a longitudinal study.

TNF-α is a pro-inflammatory cytokine[137] that can be released by adipocytes as well,[138] in addition to other cells such as vascular smooth muscle cells, endothelial cells, monocytes, and macrophages,[110] as previously noted. TNF-α plays a key role in the mediation of the immune response regulation of inflammation, cell apoptosis and survival, cytotoxicity, and the production of other cytokines such IL-6.[137] TNF-α plays an important role in insulin resistance, acting directly on the insulin receptor. Additionally, TNF-α has been positively associated with adiposity, particularly visceral fat and other

cardiovascular-disease risk factors. It also indirectly mediates lipolysis and augments the hepatic synthesis of fatty acids, thereby increasing serum levels of fatty acids and triglycerides.[138]

The adipose tissue express TNF-α constitutively,[124, 137, 139-141] especially on infiltrated macrophage,[140, 141] and its expression decrease after weight loss;[124, 139, 141] this also happen with serum TNF-α, which is correlated between body mass index and decreased after weight loss in obese adults.[137, 139, 141] In younger individuals, the relationship between TNF-α levels and body fatness measures is conflicting, according to the studies compiled on Table 1. On the one hand, several studies found a positive relationship between TNF-α and body mass index[132, 142] or waist-to-height ratio,[132] including studies in children[132] and adolescents;[132, 142] On the other hand, other researchers found no association between body mass index and TNF-α in adolescents at different ages.[107, 133-135] In addition, the sum of skinfold measurements was not associated with TNF-α levels in adolescents with a mean age of 13.4 years.[127]

TNF-α expression is increased in the adipose tissue of people with insulin resistance.[137, 139-141] Unlike the case with studies of adult, widespread systemic inflammation, including TNF-α, is not necessarily associated with insulin resistance among adolescents.[143] In fact, a studied conducted with adolescents and adults found no relationship between HOMA-IR and TNF-α in adolescents and surmised that some dissociation may exist between low-grade inflammation and insulin resistance in the early phases of obesity.[144]

Both IL-6 and TNF-α can affect liver production of lipids;[145] they inhibit lipoprotein lipase and stimulate lipolysis.[123, 137] The effects of these cytokines on triglyceride metabolism might further impair endothelial health, raising circulating concentrations of non-esterified fatty acids[123] and stimulating the release of very-low-density lipoproteins, which can become oxidized to low-density lipoprotein.[137, 145] However, the results of studies conducted with adolescents do not agree regarding the role of IL-6 and TNF-α on impaired serum lipids profiles (Table 1). On the one hand, high-density cholesterol was negatively associated with TNF-α but not with IL-6, and triglycerides were not associated with either in adolescents ages 10–14 years;[145] on the other hand, in

adolescents ages 12–18 years, none of the above associations were observed.[126] In addition, serum TNF-α was positively correlated with triglycerides and negatively correlated with high-density lipoprotein cholesterol; no correlation was found with total cholesterol, in adolescents ages 15–16 years.[146]

4.2.3.3. Adipokines

Adipokines are proteins produced mainly by adipocytes such as leptin and adiponectin. They are primarily produced by adipocytes and can therefore be properly classified as adipokines.[124]

Leptin is a protein encoded by the Ob gene, and its primary role is control of appetite. Leptin's role in regulating inflammation is to stimulate cytokine production from T lymphocytes. Leptin deficiency is associated not only with reduced inflammation in models of autoimmune disease but also with increased susceptibility to bacterial and viral infections. Therefore, the consensus is that leptin exerts a pro-inflammatory role while at the same time protecting against infection.[124]

Adipocytes are the most important source of leptin, and circulating leptin levels correlate directly with adipose tissue mass.[124] In adolescents, several studies showed a positive relationship between leptin levels and body fatness, as shown in Table 1, especially for body mass index but also for measures of abdominal fatness, such as waist circumference,[147, 148] and waist-to-hip ratio.[118, 149]

In the contrary to leptin, adiponectin appears to act as an anti-inflammatory molecule, by inhibiting of TNF-α and IL-6. Serum adiponectin levels do not increase with obesity as leptin levels do; there is a tendency for the reduction of adiponectin levels in obese subjects and increased levels in patients with anorexia nervosa.[124] Most studies have also demonstrated this relationship in adolescents (Table 1). Measures of whole-body fatness are the most studied especially body mass index,[126, 142, 148, 150, 151] but also percentage of body fat.[149] Central obesity such as waist circumference[152] and waist-to-height ratio,[118, 149] and intramyocellular lipid content[153] have also been studied.

Adiponectin has a role in the regulation of insulin sensitivity. In fact, patients with type 2 diabetes have a low level of adiponectin.[124] Adiponectin acts

through multiple tissues to enhance insulin sensitivity; thus, adiponectin is referred to as an insulin sensitizer.[140] In adolescents, reduced adiponectin levels have also been observed in several measures of impaired glucose metabolism,[126, 144, 150, 152-155] as shown in Table 1, for example, high insulin levels,[144, 150, 152, 154] HOMA-IR,[126, 152] and other models of insulin resistance.[153, 155]

Furthermore, adiponectin may also play a role in the pathogenesis of early atherosclerosis, disturbing the health of arteries by disturbing endothelial function and promoting intima-media thickening in obese adolescents, mean age 13.5[113] and 12.7 years.[156] In a meta-analysis among children and adolescents, Silva et al.[114] confirmed these findings and showed that obese had lower concentrations of adiponectin and higher values of intima-media thickening compared to non-obese.

4.2.3.4. Clusters and scores of inflammatory biomarkers

Clusters or signatures of biomarkers to identify low-grade inflammation have been encouraged.[70] Several researchers have used this approach to analyze the relationship between cardiovascular risk factors and low-grade inflammation. Adabimohazab et al.,[157] in a cross-sectional study based on adolescent sample with a mean age of 17.5 years, showed a significant difference between the lean and overweight/obese groups with using a score of eight inflammatory biomarkers. Artero et al.[158] defined a score based on five inflammatory biomarkers in a cross-sectional study in adolescents ages from 12.5–17.5 years and showed that the inflammatory biomarker score was significantly higher in overweight adolescents compared to those who were not overweight.

Table 1: Inflammatory biomarkers and their association with chronic conditions and risk factors in children and adolescents

	Body fatness [a]		Impaired glucose tolerance [b]		Impaired serum lipids profile [c]		Increased blood pressure [d]		Cardiovascular disease risk scores [e]		Metabolic syndrome		Early atherosclerosis	
	A.	Ref.	A.	Ref.	A.	Ref.	A.	Ref.	A.	Ref.	A.	Ref.	A.	Ref.
CRP	⇧	(19-21, 50, 106, 107, 112, 118, 126, 127, 132, 134, 142-144, 147, 148, 150, 151, 159-163)	⇧	(20, 106, 126, 143, 160)	⇧	(20, 112, 126, 150, 160, 161)	⇧	(106, 112, 126, 161, 164)	⇧	(127, 147, 165)	⇧	(20, 50, 112, 128, 160)	⇧	(51, 113)
	⇔	(133)	⇔	(150, 162, 166)	⇔	(106, 166)	⇔	(166)			⇔	(133)	⇔	(156)
C3	⇧	(107, 118, 162, 163) girls only(135)	⇧	(162)										
C4	⇧	(107, 118, 135, 162)	⇧	(162)										
	⇔	(163)												
IL-6	⇧	(106, 126, 132, 157)	⇧	(126) girls(167)	⇧	(106, 150)	⇧	(145, 168)	⇧	(127)	⇧	(128)		
	⇔	(107, 127, 133-135, 143, 147, 150, 169)	⇔	(106, 150, 162, 166, 168)	⇔	(126, 145, 166, 168)	⇔	(106, 126, 133, 166)	⇔	(147, 165)	⇔	(133)		
TNF-α	⇧	(132, 142, 150, 157, 169, 170) ow girls(149)	⇧	(144)	⇧	(106, 144, 145)			⇧	(127)	⇧	(128)		
	⇔	(106, 107, 126, 127, 133, 135, 143, 144, 147)	⇔	(106, 126, 150, 166, 168)	⇔	(126, 150, 166, 168)	⇔	(106, 126, 133, 166, 168)	⇔	(147, 165)	⇔	(133, 170)		
Leptin	⇧	(118, 126, 133, 142-144, 147, 148, 150, 153, 154, 170) ow girls(149)	⇧	(126, 144, 150, 166)	⇧	(126, 144, 150, 166)	⇧	(126, 133, 166)	⇧	(147)	⇧	(133)		
			⇔	(154)							⇔	(170)		
Adipo-nectin	⇩	(126, 133, 142, 144, 148, 150-155, 157, 168, 170) girls(118) ow girls(149)	⇩	(126, 144, 150, 152-155)	⇩	(126, 144, 150, 153, 155, 168) ow (152)	⇩	(126, 152, 164, 168)	⇩	(127)	⇩	(133, 152, 170)	⇩	(113, 156)
	⇔	(143, 147, 150)					⇔	(133, 155)	⇔	(147)	⇔	(155)		

Abbreviations: A., association; Ref., reference of studies; CRP, C-reactive protein; C3, complement component 3; C4, complement component 4; TNF-α, tumor necrosis factor; ow, overweight.
Symbol: ⇧, positive relation; ⇩, negative relation; ⇔, no relation.
[a] Body fatness measures could be body mass index, waist circumference, waist to height ratio, body fat percentage, skinfolds, and/or ponderal index (weight/height^3).
[b] Impaired glucose tolerance could be higher levels of serum glucose, serum insulin, homeostasis model assessment of insulin resistance (HOMA-IR), and/or whole body insulin sensitivity index.
[c] Impaired serum lipids profile could be higher levels of serum triglycerides, total cholesterol and low-density-lipoprotein cholesterol and/or lower levels of high-density-lipoprotein cholesterol.
[d] Increased blood pressure could be higher measurements of systolic and/or diastolic blood pressure.
[e] Several different scores of cardiovascular disease risk have be performed including, HOMA-IR, waist circumferences, triglycerides, systolic blood pressure and high-density-lipoprotein cholesterol; ratio of total cholesterol to high-density-lipoprotein cholesterol, triglycerides, HOMA-IR, systolic blood pressure, skinfold, and inverse of volume of oxygen peak.

4.2.4. Determinants of inflammation

A number of factors, in addition to the presence of a direct inflammatory trigger, have been shown to influence the concentrations of inflammatory biomarkers. These include sex, genetics, age, body fatness, smoking, physical activity, sedentary time, diet, gut microbiota, medications, pollution, emotional stress, and sleep pattern behavior. It has been advised that studies aiming to assess low-grade inflammation consider including these factors between the covariates.[70, 100]

An understanding of how these factors can determine basal levels of inflammatory biomarkers at an early age is very important considering two fundamental aspects: 1) the inflammatory process may play a significant role in the development of chronic conditions and their risk factors such as overall[19-21] and central[106, 107] obesity, metabolic syndrome,[20, 50] impaired endothelial function and early-stage atherosclerosis,[51] diabetes,[13, 14] cardiovascular diseases,[16, 18] and cancer;[101, 102] 2) these problems and their risk factors often have their origins at an early age, tracking from childhood to adulthood.[26-31] Therefore, understanding each of the factors that can determine basal levels of inflammatory biomarkers among adolescents is of considerable interest for the prevention of future disease while there is still an opportunity to reverse any damage.[10]

In adults, better studied determinants of low-grade inflammation include age,[104, 105] body fat,[171] sedentary time,[172] and smoking[173] as pro-inflammatory factors; and moderate-to-vigorous physical activity as an anti-inflammatory factor.[174] In regard to dietary and nutritional intake, the impact on inflammatory biomarkers can be pro- or anti-inflammatory,[69, 70] depending on the dietary and nutritional context.

In addition, other factors can play a role in low-grade inflammation. Low socioeconomic status was associated significantly with elevated levels of CRP,[175-177] IL-6, and TNF-α[176] compared to high socioeconomic status, in adults[175, 177] and seniors.[176] This association between socioeconomic status and levels of CRP is also observed in children[178] and adolescent,[54-56]

specifically low parent education, and adiposity explains in part this association.[54, 56]

In fact, body fatness is the most widely studied determinant of low-grade inflammation among adolescents. Several large-scale studies[19-21] have demonstrated that healthy young subjects with more body fat have moderately higher concentrations of inflammatory markers than their leaner peers, supporting the concept that obesity should be considered a state of low-grade inflammation. Less data are available to allow us to elucidate how physical activity or dietary and nutritional intake may have a direct effect on inflammation in apparently healthy, disease-free young populations.[10]

In fact, this gap in the literature has been recognized and considered a challenge for examining the relationship between dietary and nutritional intake and inflammatory biomarkers in healthy young populations and partially explains the scarcity of published research.[10] Beyond the role of adipose tissue on inflammation and its relationship to inflammatory biomarkers in adolescents, the following topics will address a brief review about the current knowledge of the roles of physical activity and dietary and nutritional intake on low-grade inflammation in adolescents.

4.2.4.1. Body fatness

Adipose tissue is not only an energy-storage organ; it is also an active participant in regulating physiologic and pathologic processes, including immunity and inflammation. Adipose tissue is comprised mainly of adipocytes but also by macrophages, present in stromal vascular fraction, and their number is directly correlated with adiposity and with adipocyte size.[124]

Adipose tissue expresses and secretes into the systemic circulation a growing list of pro- and anti-inflammatory biomarkers, including IL-6, TNF-α and leptin, and adiponectin.[69, 124] Macrophages are components of adipose tissue and actively participate in its activities.[124] The anatomical localization of adipose tissue seems to have a paramount importance in relation to its physiological function.[69]

Increased abdominal fat mass is associated with an elevation of the circulating concentrations of inflammatory biomarkers including several acute-phase inflammatory proteins such as CRP, adipokines, and cytokines. The adipokines are distinct between the subcutaneous and abdominal adipose tissues: leptin is preferentially expressed and secreted by subcutaneous adipose tissue, while the expression of adiponectin is higher in abdominal fat. The cytokines IL-6 and TNF-α seem to be equally synthesized by the different sites. Deposits of adipose tissue at ectopic sites, such as the liver, heart or skeletal muscle may contribute to the production of inflammatory mediators in the absence of obesity.[69]

Contrary to what happens with adults, the adipose-tissue biopsies in overweight children showed a lower expression of adiponectin in the visceral adipose tissue compared to subcutaneous adipose tissue; in normal-weight children, no difference in adiponectin expression between fat deposits was seen.[179] In fact, although there is a negative association between body fatness measures such as body mass index or skinfold measures and adiponectin, the same is not true for abdominal fatness measures; several authors found no relationship between adiponectin and waist circumference[118, 143, 144, 147] or between adiponectin and abdominal fat[142] in adolescents. However, waist-to-height ratio was negatively associated with adiponectin but only in girls.[118]

Actually, sex differences have been observed and can play a role in the modulation of the relationship between low-grade inflammation and obesity. Ford et al.[180, 181] have studied the CRP concentration in several large samples and concluded that, in adulthood, women typically have higher mean CRP than men.[180] These data were confirmed by a meta-analysis,[182] but sex differences observed in adults were not found in children. However, Ford et al.[181] have suggested that these differences emerge only after age 15. In a longitudinal study of sex differences in relation to CRP from childhood to young adulthood, Shanahan et al.[183] found that sex-differentiated increases in CRP during adolescence were, in part, accounted for by increasing body mass index and CRP associations in girls. In concordance, Ahonen et al.[184] showed that a relative increase in weight from youth to middle age may be more harmful in women than in men with respect to inflammatory biomarkers. Pirkola et al.[185] found in

a prospective analysis that being small for gestational age at birth (a risk factor for obesity in adulthood[186]) was associated with a pro-inflammatory state in girls during adolescence, but this relationship was not observed in boys. In addition, intrauterine exposure to maternal overweight was associated with elevated low-grade inflammation in girls, as was exposure to paternal overweight during the fetal period in boys.

However, as can be observed in Table 1, measures of body fatness of adolescents in general are positively associated with a state of low-grade inflammation assessed by high CRP, C3, C4, and leptin, or low adiponectin, although the findings about the relationship between IL-6 and TNF-α and body fatness are inconsistent. Furthermore, persistent low-grade inflammation in obese children may increase the risk of metabolic and cardiovascular events in later life.[179] In addition, low-grade inflammation later in life can be established as early as at birth since maternal body mass index at delivery predicted newborns' plasma (umbilical cord plasma) levels of CRP, IL-6, and TNF-α.[187]

4.2.4.2. Physical activity

Large cohort studies consistently show an inverse association between physical activity and low-grade inflammation, and its impact is most evident when physical activity is more frequent and more intense. Data from intervention studies has also suggested that accumulated physical activity training can reduce chronic low-grade inflammation.[174]

In adolescents, data from cross-sectional studies support the association between increased levels of physical activity and decreased low-grade inflammation.[188] Authors found that physical activity was inversely associated with CRP, C3, C4,[189] and IL-6[190] in adolescents ages 12.5–17.5 years old from 10 European cities[189] and with in 12-year-old French.[190] Moreover, fitness level was negatively associated with CRP in adolescents ages 17 years old.[191] In addition, muscle strength was negatively associated with CRP, C3,[158, 192] C4, and an inflammatory score[158] in adolescents ages 13 to 18.5[192] and 12.5 to 17.5 years.[158] However, other researchers found that physical activity was not independently associated with inflammatory biomarkers in prepubertal children

and adolescents ages 9-10[163] and 13–17 years,[162] suggesting that physical activity plays an indirect role in low-grade inflammation.[162, 163]

In contrast, overtraining can also increase inflammatory biomarkers.[188] Athletes undergoing an intensified period of training without opportunity for sufficient rest can experience what is known as the overtraining syndrome. This syndrome is at least partially mediated by low-grade inflammation, although it is not fully understood, especially in relation to augmented circulating levels of pro-inflammatory cytokines such as IL-6 and TNF-α.[174]

In regard to the amount of time spent in sedentary activities, this has been positively associated with low-grade inflammation in adults,[174, 193, 194] independently of the amount of time spent in moderate-to-vigorous physical activities.[195] This relationship is particularly important because sedentary behavior is correlated with health risk independent of physical activity.[172] In adolescents, the data are not so convincing. Martinez-Gomez et al.[196] showed that television-viewing time but not total sedentary time was positively associated with measures of adhesion molecules but not with CRP, C3, C4, IL-6, adiponectin, or leptin. In addition, two other investigations studied the relationship between screen time and CRP in adolescents.[185, 197] While Pirkola et al.[185] found an association only for girls in the unadjusted model, Hardy et al.[197] found an association only for boys in the unadjusted model. However, these associations were attenuated[185] or lost[197] in adjusted analyses, respectively.

4.2.4.3. Dietary and nutritional intake

Dietary and nutritional intake are considered important regulatory factors for immune response since optimal nutrition is required for a healthy immune balance: on the one hand, malnutrition leads to immunosuppression; on the other hand over-nutrition increases the susceptibility to an inflammatory condition.[198] Several individual nutrients, bioactive food components, whole foods, and dietary patterns have been identified as modulators of inflammation.[69, 70, 100]

Nutritional intake and bioactive food components

Calder et al.[69] performed a review of the major contributors and the most relevant nutrients and bioactive food components for low-grade inflammation in studies conducted especially in adult samples. Very little is known about nutrient and bioactive food component intake and low-grade inflammation in adolescents.

In regard to fat intake, dietary fatty acids can influence low-grade inflammation through the modulation of eicosanoid metabolism and the regulation of cell membranes.[69] In addition, genes involved in inflammatory processes may interact with environmental exposure such as dietary fatty-acid intake, in particular SFA, n-6, and n-3 PUFA by having an impact on the expression of cytokine genes.[199] In reviews, several authors have considered polyunsaturated PUFA, especially n-3, as protective for low-grade inflammation, while SFA has been considered to promote inflammation.[69, 199] Nevertheless, controversy exists about the association between fatty acids and inflammation, especially in data from adolescent-based studies.

In regard to SFA, Santos et al.,[200] based on a systematic review of the role of SFA intake on low-grade inflammation in adult populations, concluded that a potential positive association between SFA and CRP but not with adipokines is suggested. Aeberli et al.,[166] in agreement with adult-based data, showed that SFA intake was positively associated with a higher level of CRP but not with IL-6 and leptin among children and adolescents (ages 6–14 years). In addition, in a cross-sectional study in youth (ages 14–25 years), Arya et al.[201] found that SFA intake was positively associated with a higher level of CRP in an unadjusted model, but the statistical significance was lost after adjustment. In contrast, Wang et al.[202] found the opposite relationship between low-grade inflammation and specific SFA when measured in serum levels of phospholipids in a cross-sectional analysis of adolescents (mean age 15 years). These authors concluded that a higher serum level of phospholipid pentadecanoic acid (15:0) and heptadecanoic acid (17:0), both dairy fatty acids, was associated with lower CRP only among overweight and lower IL-6 independent of weight status but not for TNF-α and adiponectin. In addition, in a cross-sectional study in children (ages

2–9 years), Gonzalez-Gil et al.[203] found sexes differences in the relationship between whole-blood fatty acids and CRP; while the sum of n-6 and LA were associated with a lower CRP level in boys, n-6 highly unsaturated, AA, and AA/LA ratio were associated with a higher CRP level in girls. These findings should be compared with other studies with caution since data have different origins, dietary fat, and serum fat.

Considering PUFA during adolescence, Klein-Platat et al.[204] measured the plasma fatty acids composition in phospholipids and cholesterol ester fractions in 12 year olds and found that the PUFA/SFA ratio was inversely associated with IL-6 in both fractions. In addition, CRP was inversely associated with two of the n-3 PUFA, ALA and EPA, in cholesterol ester fractions. Considering data from dietary intake, Arya et al.[201] found no association between total PUFA, n-6, n-3, or n6/n3 ratio and CRP in a sample of adolescents and young adults (14–25 years old). By contrast, Aeberli et al.[166] showed a positively association between PUFA and CRP but not for IL-6 and leptin in a study of children and adolescents ages 6–14 years old. The controversy regarding the findings by Aeberli et al.[166] may reside in the fact that n-3 and n-6 PUFA are measured together, and the n-6 PUFA class of fatty acids can act in both a pro- and anti-inflammatory manner.[199]

EPA and DHA are able to partly inhibit a number of aspects of inflammation, including production of eicosanoids from the n-6 PUFA, but also leukocyte chemotaxis, adhesion molecule, and inflammatory cytokines.[205] The role of ALA in reducing low-grade inflammation has been attributed to the potential of ALA to be a substrate for the synthesis of EPA and DHA and so to increase the serum concentrations of EPA or EPA+DPA and the content of EPA in mononuclear cells, such as neutrophils and monocytes.[206]

Dietary carbohydrates and fiber may also play a role in inflammation. Observational studies suggested that there is a positive association between glycemic index and glycemic load and low-grade inflammation,[69, 70] and a negative association between dietary fiber and low-grade inflammation.[66] However, intervention studies do not support these associations consistently.[66, 69, 70]

Kuo[207] in a review assigns the role of dietary fiber on prevention of low-grade inflammation for the presence of body weight-related and body weigh-unrelated anti-inflammatory activity of fibers. The low energy density of a fiber-rich diet likely contributes to the body weight–related anti-inflammatory activity by curtailing the obesity-induced chronic inflammation. The fermentability of fiber and the consequent changes in the intestinal microbiome and/or their metabolites likely lead to the body weight–unrelated anti-inflammatory activity locally in the intestine and systemically. In fact, Authors[208, 209] showed a difference in the gut microbiota composition of bacterial species between obese and normal weight children and adolescents.

Two trial studies in samples of pediatric-age subjects[210, 211] did not also provide strong support for a relationship between different types of carbohydrates and low-grade inflammation. On the one hand, Iannuzzi et al.[210] found a decrease in CRP in children and adolescents ages 7–13 years after following a low-glycemic hypocaloric diet for 6 months. However, CRP also decreased in high-glycemic hypocaloric diet groups, and therefore, the effect of glycemic index on the CRP biomarker is unclear. On the other hand, Rouhane et al.[211] found a decrease in CRP and IL-6 but not adiponectin in a group of obese girls ages 12–18 years who received recommendations for a low-glycemic-index diet for a 10-week period. However, IL-6 also decreased for the control group that received general recommendations for a healthy diet. In fact, only CRP and not IL-6 and adiponectin was lower in the low-glycemic-index group compared to the healthy diet group after intervention. However, this association was marginally significant ($p=0.08$).

In a prospective study, a higher dietary glycemic load but not dietary fiber intake, during puberty (9–15 years) predicted greater IL-6 concentrations in young adults (18–36 years). No relationship was found between the quality measurements of several carbohydrates and CRP or adiponectin.[212] In contrast, dietary fiber intake was negatively associated with CRP and positively associated with adiponectin but not with leptin in a cross-sectional study conducted with adolescents ages 14–18 years.[213]

The antioxidant vitamins, such as vitamin C, vitamin E, and the carotenoids, have been proposed as playing a role in low-grade inflammation.[69] Dietary antioxidants are substances in foods that significantly decrease the adverse effects of reactive species, such as reactive oxygen and nitrogen species, on normal physiological functioning in humans.[62] In a review based mainly on adult samples, Calder et al.[69] observed that, although intervention studies using supplements of those nutrients either alone or in different combinations did not provide consistent evidence of this relationship, observational studies provided a consistent picture of an anti-inflammatory effect of antioxidant vitamins. Three cross-sectional studies in pediatric-age subjects confirm that evidence,[166, 214, 215] although inflammatory biomarkers were not associated with vitamins in a consistent manner. Holt et al.[214] found that vitamin C intake was correlated with low levels of CRP and IL-6 but not with TNF-α, and intake of β-carotene was associated with low levels of IL-6 and TNF-α but not with CRP in adolescents ages 13–17 years. Aeberli et al.[166] demonstrated that the intake of vitamin C, vitamin E, and β-carotene was associated with high leptin levels but not with CRP or IL-6 in children and adolescents ages 6–14 years old. And finally, García et al.[215] concluded that vitamins A, C, and E were negatively correlated with CRP in children ages 6-10.5 years old. In addition, interaction analysis showed that children who were overweight and obese and also had low concentrations of vitamin A had higher CRP.

Bioactive food components are phytochemicals present in plant foods, especially in the pigmentation, and these are likely to be protective for low-grade inflammation when consumed as plant-based foods and not supplements.[69] The most studied of the phytochemicals are the polyphenols and carotenoids. Flavonoids are the most abundant polyphenols present in the human diet, and they can be divided into several classes including flavonols, flavones, flavanols or catechins, flavanones, anthocyanidins, and isoflavonoids.[216] It is difficult to determine the exact component or components that would be effective for reducing inflammation biomarkers because few studies have investigated the effects of an isolated bioactive compound. In fact, the food matrix is accompanied by several polyphenols that can exert additional synergistic functions; this limits the conclusions that can be drawn regarding the compounds that actually do

prevent low-grade inflammation.[217] Studies investigating the effect of flavonoids on inflammatory biomarkers are insufficient and are focused mainly on flavonoid-rich foods rather than on pure molecules;[218] however, evidence suggests that higher intakes of selected flavonoids are associated with modestly lower concentrations of several inflammatory biomarkers.[69] Aligning with the review by Calder et al.,[69] Holt et al.,[214] in a cross-sectional study of adolescents (ages 13–17 years), studied the correlation between total and five individual flavonoid intakes (three flavanols: kaempferol, myricetin, and quercetin; and two flavones: apigenin and luteolin,), consumed as foods and levels of CRP, IL-6, and TNF-α. Only luteolin was negatively correlated with TNF-α, after adjusting for confounders.

In fact, plant-based foods are recognized as rich in bioactive phytochemicals,[216] including those that have been widely studied such as flavonoids and carotenoids, and other unknown bioactive components that could be beneficial either alone or in combination.[219] Those bioactive compounds are recognized as having an anti-inflammatory effect, although their mechanisms remain poorly understood;[220] thereforethe the exact components and their dosages for supplementation purposes to reduce inflammation have not yet been identified.[217] Unlike supplementation, the food matrix is accompanied by a variety of substances that can exert additive and synergistic functions,[217] so the beneficial effects of those bioactive components and their diversity can also be attributed to additive and synergistic effects responsible for their potent antioxidant activities.[221]

Selenium is considered an antioxidant mineral and its biological functions include defense against oxidative stress, regulation of thyroid hormone action, and regulation of the redox status of vitamin C and other molecules.[62] Selenium intake may also play a role on reducing inflammation,[222] however, studies in adolescents did not also provide strong support for this relationship. On the one hand, Del Mar Biblioni et al.[223] found that dietary selenium intake was correlated with low levels of leptin in a cross-sectional study in girls (ages 12–17 years); on the one hand, Murrer et al.[224] found no relationship between

selenium supplementation and CRP, IL-6, and leptin in a randomly trial study performed in overweight or obese adolescents (ages 10–18 years).

García et al.[215] also studied the relationship between several minerals and low-grade inflammation, and iron but not zinc was negatively correlated to CRP in a cross-sectional study of children ages 6–10.5 years. However, in adjusted linear regression, this association was not seen. Two cross-sectional studies[225, 226] found controversial results regarding the relationship between iron and low-grade inflammation, considering CRP, in overweight and obesity. While Tussing-Humphreys et al.[225] considered that overweight girls (mean age 14.5 years) have a greater risk for iron deficiency and that inflammation contributes to this, Ferarri et al.[226] concluded that the adiposity of the adolescents (mean age 14.9 years) was sufficient to cause inflammation but not sufficient to impair iron status and cause iron deficiency.

Finally, magnesium may also play a role in potentiating inflammatory processes, by inhibiting proliferation and migration of vascular smooth muscle cells and macrophages and promoting plaque stabilization and regression.[227] Several adult-based studies[228-231] reported a relationship between low intake or serum magnesium and high CRP level. In accordance, mostly adolescent-based studies show the same relationship. Lopez-Alacón[232] showed a negative relationship between magnesium intake and levels of CRP in adolescents, mean age of 12 years; Rodríguez-Morán and Guerrero-Romero[233] showed an association between low serum magnesium and high CRP levels in adolescents ages 10-13 years;[233] and King et al.[234] reported that adolescents and children (ages 6–17 years) with intakes below the recommended dietary allowance are more likely to have elevated CRP levels. However, in contrast to previous studies, Del Mar Bibiloni[223] showed that magnesium was positively associated with IL-6 and no association with adiponectin and leptin, in girls (ages 12–17 years).

Whole foods and food groups

The intake of whole grains as opposed to refined grains was described as inversely associated with markers of low-grade inflammation in observational studies.[235-237] Intervention analysis provided conflicting results.[238-240] Although the mechanisms of this relationship are not precisely known, it is

believed that dietary fiber, vitamins, minerals, and phytochemicals are involved. The effect or association was measured especially for CRP but also for IL-6 in adult-based samples.[69] In a pediatric sample, a randomized crossover clinical trial showed an effect of high whole-grain intake compared with low intake on decreasing CRP and leptin serum after 6 weeks in overweight or obese girls ages 8–15 years.[241]

Fruits and vegetables seem to be negatively associated with low-grade inflammation when treated as food groups together or separately in longitudinal [242] cross-sectional,[214, 243-255] and intervention[256, 257] studies based on adult samples. According to Calder et al,[69] when the focus was on a single variety of fruit or vegetable, interventional studies reported inconsistent results. Not only the quantity but also the variety of fruit and vegetables seems to be important; Bhupathiraju and Tucker[243] found an association between fruit and vegetable variety and low-grade inflammation in the elderly. In adolescents, Holt et al.[214] found that a high intake of fruits was correlated with low levels of CRP and IL-6 but not with TNF-α; high vegetable intake was correlated with low levels of IL-6 and TNF-α but not with CRP; and finally, high intake of fruits and vegetables treated together as a single group was correlated with low levels of all inflammatory biomarkers in studies conducted with adolescents ages 13–17 years.

Studies have assessed the relationship between fish intake and low-grade inflammation. The observational studies in adults[258-261] have not yet met with consensus, while two studies found an inverse relationship between fish consumption and CRP,[258, 259] IL-6, and TNF-α.[258] Two other studies[260, 261] did not find any relationship with CRP.[69] Studies of subjects of pediatric age are scarce and inconclusive. A cross-sectional study of children and adolescents ages 9–11 years old assessed fish consumption, total blood mercury, and phase-acute proteins. Subjects who consumed fish had higher levels of blood mercury. A high level of mercury was associated with several acute-phase proteins including C3.[262] Controversially, another study[263] reported that a higher level of blood mercury was associated with lower levels of TNF-α but not with cytokines. The authors did not directly report the relationship between fish consumption and

acute-phase proteins or cytokines, and they reported mercury levels well below the potential health risk level.

The regular consumption by adults of beverages containing alcohol, such as wine or beer, has been reported in a review by Calder et al.[69] to be inversely associated with low-grade inflammation, where several studies reported a U-shaped relationship. A moderate daily intake of 1 to 2 drinks/day was often found to be associated with decreased concentrations of inflammatory biomarkers, especially for CRP.[69] In youth, a positive relationship between alcohol and low-grade inflammation is observed but is not reported in a U-shaped manner.[264, 265] In a cross-sectional study conducted among college students, the authors found that a session of heavy drinking of alcoholic beverages was associated with increased mean plasma TNF-α in males and a decrease in females, compared to controls.[264] In agreement, a prospective analysis showed that CRP levels were significantly higher in youth who used alcohol among children, adolescents and young adults ages 9–12, 13–16, and 19–21 years.[265]

Considering milk and dairy products, in a cross-sectional study in healthy adults, Panagiotakos et al.[266] found that dairy consumption of 11–14 portions/week is associated with decreased levels of CRP, IL-6, and TNF-α levels compared to fewer than 8 portions/week. Considering intervention studies, reviews about relationship between dairy intake and low-grade inflammation in adults have been developed,[267, 268] but conclusions are contradictory. Labotene et al.[267] reviewed 8 studies and considered the data insufficient to suggest a beneficial or neutral impact of dairy on inflammation. Bordoni et al.[268] reviewed 52 clinical trials and developed an inflammatory score to measure low-grade inflammation. The authors concluded that dairy products might induce an anti-inflammatory effect, even for subcategories of dairy products: low fat, high fat, and fermented; this anti-inflammatory effect was stronger in persons with metabolic disorders, but a pro-inflammatory activity was observed in subjects allergic to bovine milk. In adolescents, studies are limited. In an intervention study about inflammatory adaptations to a short-term exercise intervention with and without milk intake in obese adolescents, Liu et al.[269] assessed a daily intensive exercise protocol

over one week and observed an elevation in TNF-α and no change in CRP or IL-6, and no differences between the milk group and the control group.

Finally, nut intake and low-grade inflammation have also been studied in adult samples.[254, 270-272] Salas-Salvadó et al.[270] in a review study, Jiang et al.[271] in a cross-sectional study, and Salas-Salvadó et al.[254] in a longitudinal study but not Ros et at.[272] in a intervention study agreed that frequent nut consumption is associated with lower concentrations of several inflammation biomarkers including CRP and IL-6. Data about adolescents are limited, but in agreement with adult data. In a randomized trial, Maranhão et al.[273] provided an amount of 15–25 g/day of Brazil nuts during 16 weeks to obese girls (mean age of 15.4 years) and found no effect on CRP when compared with a supplementation placebo.

Dietary patterns

Approaches based on dietary patterns examine combinations of foods and nutrients. Taking into account that nutrients or foods are rarely eaten in isolation, dietary patterns consider synergistic or antagonistic interactions among foods components,[274] and may provide an advantageous approch. Dietary pattern research is generally based on two kinds of methods: a priori using diet scores or a posteriori using data-driven techniques such as factor analysis and cluster analysis.[69, 275]

According to reviews by Barbareskoet al.[275] and Ahluwalia et al.,[276] most of the cross-sectional studies in adults[277-281] support a positive association between a Western dietary pattern using a posteriori techniques and inflammatory biomarkers. In adolescents, Del Mar Biblioni et al.[223] found a positive association between a Western dietary pattern and IL-6 but not with CRP, TNF-α, leptin, and adiponectin in girls ages 12–17 years.

A Mediterranean-style diet can be characterized as a dietary pattern rich in fruits, vegetables, whole grains, pulses, nuts, fish, and low-fat dairy products. It includes moderate amounts of wine and olive oil as a main source of fat.[69] Reviews of the results of adult-based studies,[69, 275, 276, 282] including cross-sectional[283-285] and intervention studies[286-288] strongly suggest that a Mediterranean-style diet can lead to reductions in low-grade inflammation,

especially in relation to CRP and IL-6. A meta-analysis[289] from randomized trials confirms that association and the beneficial effect on reducing CRP and IL-6 and increasing adiponectin. Antagonistically, another study did not find an association between a Mediterranean-style diet and CRP, IL-6, TNF-α, leptin, and adiponectin in girls ages 12–17 years.[223]

Healthy Eating Index (HEI)[290] is a dietary score based on dietary guidelines and was inversely associated with an elevated CRP[235, 283, 291] and IL-6,[283, 292] in studies conducted among adults including a population-based study.[235] The Alternative Healthy Eating Index (AHEI)[293] is other dietary indices HEI-adapted and was also inversely associated with an elevated CRP[283] and IL-6[283] in cross-sectional[283] and longitudinal[294] studies conducted in adults.

Considering other index, the Dietary Approaches to Stop Hypertension (DASH)[295] was negatively associated with low-grade inflammation in adults.[296] In adolescents, adherence to the DASH diet, compared to the usual dietary advice, had a significant effect on serum CRP levels but not on IL-6, TNF-α, and adiponectin after 6 weeks of an intervention among girls ages 11–18 years.[297]

The Dietary Inflammatory Index (DII) was developed to measure the inflammatory potential of diet in adults. DII is a literature-based tool that was designed by Cavicchia[298] and updated by Shivappa.[299] It scores an individual's diet in a range from -8.87 to +7.98, and it is interpreted as strongly anti-inflammatory to strongly pro-inflammatory, respectively.

The DII was developed based on reviews about the role of food and dietary constituents on the following inflammatory biomarkers: CRP, TNF-α, and the interleukins -1β, -4, -6 and -10. The review pointed to 45 food parameters as nutrients and bioactive food components (alcohol, vitamin B12, vitamin B6, β-carotene, caffeine, carbohydrate, cholesterol, energy, eugenol, total fat, fiber, folic acid, garlic, ginger, iron, magnesium, MUFA, niacin, n-3 PUFA, n-6 PUFA, onion, protein, PUFA, riboflavin, saffron, saturated fat, selenium, thiamin, TFA, turmeric, vitamin A, vitamin C, vitamin D, vitamin E, zinc, green/black tea, flavan-3-ol, flavones, flavonols, flavonones, anthocyanidins, isoflavones, pepper, thyme/oregano, rosemary). These were scored as +1, -1 or 0, according to their inflammatory effects: pro-, anti-, or null, respectively. Antioxidant vitamins,

several flavonoids, dietary fiber, and n-3 PUFA are the parameters included in the DII that contribute to a more anti-inflammatory score. Total fat, SFA, and TFA are the parameters that contribute to a more pro-inflammatory score. Shivappa et al.[299] described an extensive list, including scores for food components that can have an effect on inflammation.

This index was initially (in its first version) associated with CRP in apparently healthy adults[298] and seniors[300] and also linked to other inflammation biomarkers such as CRP, IL-6, and a combined inflammation biomarker score in adults and the elderly.[301] For the updated version, several studies found a correlation between DII score and CRP in adults and the elderly,[302] and CRP, IL-6, TNF-α, and a score of combined inflammatory biomarkers in postmenopausal women.[303]

A recent review considered the DII score useful for understanding the relationships between diet, inflammation, and cardiometabolic diseases.[304] Furthermore, the DII score has been used in several studies to predict mortality,[305, 306] survival[307], and diseases, especially cancer,[307-317] but also obesity,[318] cardiovascular disease,[319] metabolic syndrome,[318, 320] kidney function,[300] bone mineral density,[311] asthma,[321] and depression.[322]

Other dietary indices distinctly different in nature and based on dietary guidelines or specific health benefits were compared to the DII score.[323] The HEI,[290] the AHEI,[293] and the DASH[295] have been negatively associated with DII scored.[323] Wirth et al.[323] found that young adults (21–35 years old) with a lower inflammatory potential of the diet (lower scores in the DII) had healthier diets (higher scores of those indices).

In addition, in a control trial,[324] overweight and obese adults were randomized into vegan, vegetarian, pesco-vegetarian, and omnivorous diets. DII scores were lower (that is, had more anti-inflammatory diet properties) for participants who followed a vegan, vegetarian, or pesco-vegetarian diet following 2 months of intervention compared to baseline.

Another interesting comparison to the DII score was a simulation analysis of three different eating patterns: fast food, Mediterranean, and macrobiotic diets.

Steck et al.[325] indicated a model meal plan and calculated the DII score for each eating pattern and concluded that a fast-food diet has pro-inflammatory properties (DII score = +4.07), while a Mediterranean-style diet and a macrobiotic diet have anti-inflammatory properties (DII score = -3.96 and -5.54, respectively).

Recently, a dietary pattern designated as the empirical dietary inflammatory index (EDII),[326] was developed to measure the inflammatory potential of diet based on food groups. The EDII works much like the DII score; higher values implied pro-inflammatory diet properties and lower values implied anti-inflammatory diet properties. A total of 18 food groups are considered in the EDII; 9 were positively associated with EDII, and the other 9 were negatively associated.

All studies analyzed in relation to DII score were based on samples of adults or seniors. No study related to the DII score based on an adolescent sample was found.

5. Aims

It is important understand the paper of dietary and nutrition intake on low-grade inflammation. Our aims try to responds some questions in this context.

This thesis includes reports from studies with the following specific objectives:

1. **Paper I:**

To identify the main dietary fatty acids predictive of a low-grade inflammation state (measured by a set of biomarkers and an overall inflammatory biomarker score), considering others no nutritional important covariates, in adolescents.

2. **Paper II:**

To study the association between fruit, vegetable, vegetable soup, and "5-a-day" fruit and vegetable recommendation intakes with a set of inflammatory biomarkers and an overall inflammatory biomarkers score, in adolescents.

3. **Paper III:**

To study the association between fruit or vegetable variety and low-grade inflammation study the association of fruit and vegetable variety with low-grade inflammation, measured by four inflammatory biomarkers and one overall inflammatory biomarkers score, in adolescents.

4. **Paper IV:**

To study the association between the dietary inflammatory index score and inflammatory biomarkers, measured by four inflammatory biomarkers and one overall inflammatory biomarkers score, in adolescents.

6. Methods

6.1. Design of LabMed Physical Activity Study

This thesis is based on a cross-sectional analysis from baseline data (collected between October and December 2011) of the Longitudinal Analysis of Biomarkers and Environmental Determinants of Physical Activity Study (LabMed Physical Activity Study).

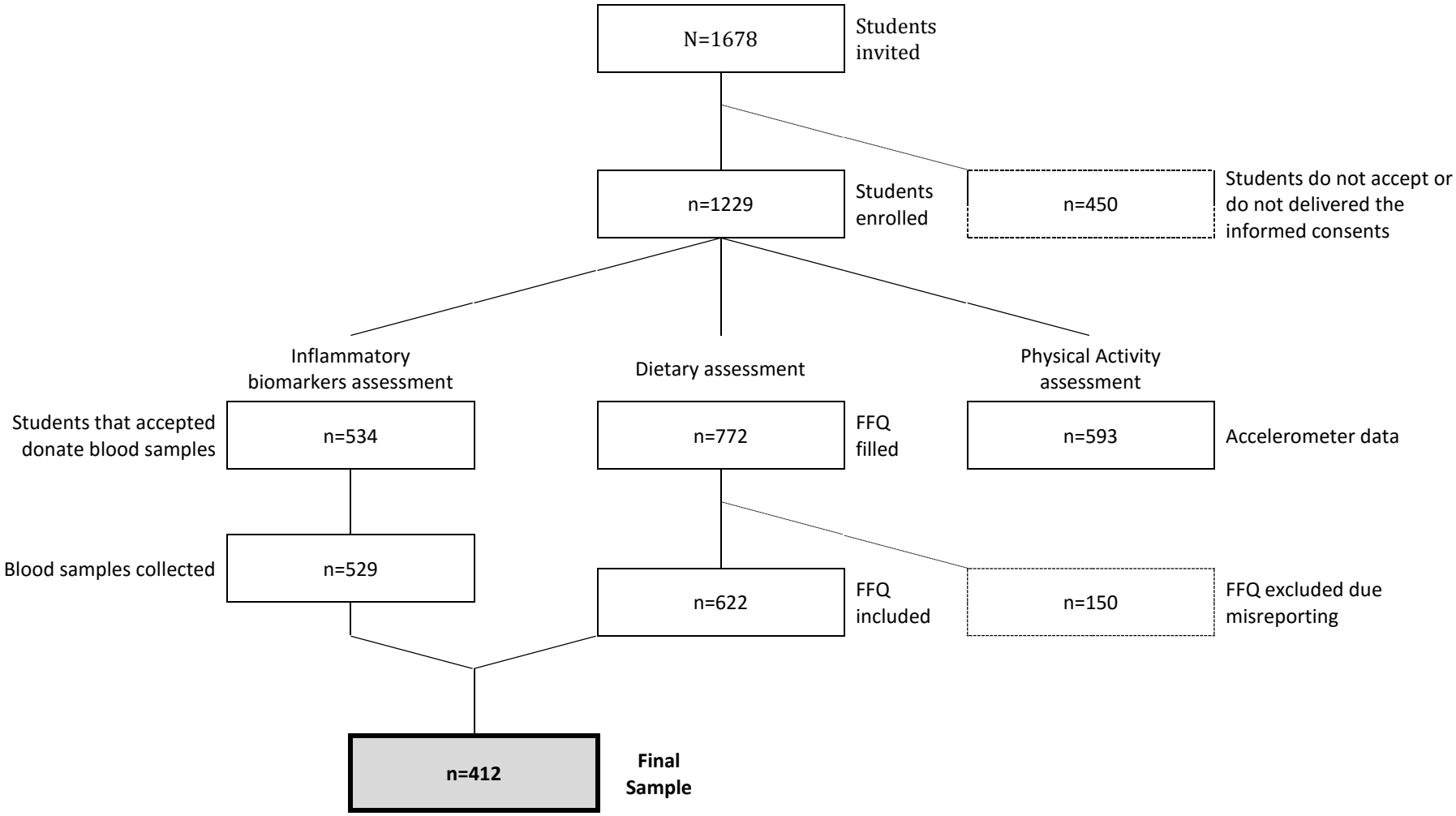
The LabMed Physical Activity Study is a school-based prospective cohort study conducted in five schools in the north of Portugal, which aim is evaluate the independent and combined associations of dietary intake and fitness levels on blood pressure levels of adolescents. The study recruitment was conducted in participating schools that have collaboration agreements previously established with the Research Centre in Physical Activity, Health and Leisure (CIAFEL).

6.1.1. Sample

A sample size was estimated for the LabMed Physical Activity study, considering a prevalence of 14% for the combined healthy diet/physical activity pattern exposure.[327] We archived to a sample size of 1086 subjects, considering a power of 80% and two-tailed significance level of 5%, to detect 15% of difference between exposed and unexposed and a dropout rate of 20%.

To fulfill the minimal sample size, all students from grades seven and ten were invited (N=1678) to participated. The criterion for participation was both the adolescents and their parents or legal guardian provided written informed consent (n=1229), although blood samples were collected only in subjects without known disease. All data were collected in schools, during the physical education classes.

For this thesis, the sample comprises 412 apparently healthy adolescents (216 girls), between 12 and 18 years old, from an initial total sample of 1229 subjects that met the following criteria: undergone blood samples collection (n=534) and filled an accurate food-frequency questionnaire (n=622) (misreported dietary assessment excluded as explained in 6.2.2 Dietary intake assessment section (page 76). The sample selection is detailed in the flowchart above (Figure 2).



Abbreviations: FFQ – food frequency questionnaire.

Figure 2: Flowchart of participants' selection for this thesis

6.1.2. Ethical and legal requirements

The LabMed Physical Activity study was conducted in accordance with the World Medical Association's Helsinki Declaration for Human Studies. The Portuguese Data Protection Authority (#1112434/2011), the Portuguese Ministry of Science and Education (0246200001/2011), and the Faculty of Sport, University of Porto, approved the study. All participants in this study were informed of the study's goals, and written informed consent was obtained from participating adolescents and their parents or legal guardian.

6.2. Variables

For this thesis, the variables included are listed in Table 2. The main variables groups are inflammatory biomarkers analyzed using blood samples, as dependents variables, and dietary and nutritional intake analyzed using food-frequency questionnaire, as independents variables. The other covariates were considered confounders and selected according to their potential influence on inflammatory biomarkers.[69, 70, 100]

6.2.1. Inflammatory biomarkers assessment and the overall inflammatory biomarker score

After at least ten hours of fasting, participants in a sitting position donated a blood sample collected from the antecubital vein. The samples were refrigerated (4º–8°C) and sent to a laboratory to determine the inflammatory biomarkers: CRP by the latex-enhanced turbidimetric assay (Siemens Advia 1600/1800, Erlangen, Germany); IL-6 by the chemiluminescence immunoassay (Immulite 2000, Diagnostic Products Corporation, Los Angeles, CA); C3 and C4 by the immunoturbidimetric assay (Siemens Advia 1600/1800, Erlangen, German).

Considering that an elevation in the concentration of inflammatory biomarkers can be minimal or absent in low-grade inflammation,[100] and no accepted international cut-points have been defined for adolescents, we have decided to consider the inflammatory biomarkers' sex- and age-adjusted median values in order to create two groups of participants: higher or lower (inflammatory state).

Table 2: Variables considered for this thesis according to papers

		Variables [a]	Papers I	II	III	IV
Dependents	**Inflammatory Biomarkers**	**CRP** (mg/dl, "higher" or "lower" values)	x	x	x	x
		IL-6 (ng/dl, "higher" or "lower" values)	x	x	x	x
		C3 (mg/dl, "higher" or "lower" values)	x	x	x	x
		C4 (mg/dl, "higher" or "lower" values)	x	x	x	x
		Overall inflammatory biomarkers scores (0-4 points, "0-1" or "2-4" biomarkers considered higher)	x	x	x	x
Independents	**Dietary and nutritional intake**	**Energy intake** (kcal/day) [b, c]	x	x	x	x
		Protein (%en)	x			
		Total carbohydrates (%en)	x			
		Sugars (%en)	x			
		Dietary Fiber (g/1000kcal)	x			
		Total fat (%en)	x			
		SFA (%en)	x			
		MUFA (%en)	x			
		PUFA (%en)	x			
		n-6 (%en)	x			
		AA (%en)	x			
		LA (%en)	x			
		n-3 (%en)	x			
		ALA (%en)	x			
		EPA+DHA (%en)	x			
		TFA (%en)	x			
		Alcohol (%en)	x			
		Vegetable (g/day, portions/day, "<3" or "≥3" portions/day) [d]		x		
		Fruit (g/day, portions/day, "<3" or "≥3" portions/day) [e]		x		
		Vegetable soup (g/day, portions/day, "<2" or "≥2" portions/day) [f]		x		
		Fruit and vegetable (portions/day, "<1" or "≥1 to <5" or "≥5" portions/day)		x		
		Vegetable variety (categories/month, tertiles)			x	
		Fruit variety (categories/month, tertiles)			x	
		DII score (-5.36 to 4.24, tertiles)				x
		Alcohol (g/day) [b]				x
		Vitamin B12 (µg/day) [b]				x
		Vitamin B6 (mg/day) [b]				x
		β-Carotene (µg/day) [b]				x
		Caffeine (g/day) [b]				x
		Total carbohydrate (g/day) [b]				x
		Cholesterol (mg/day) [b]				x
		Total fat (g/day) [b]				x
		Fiber (g/day) [b]				x
		Folic acid (µg/day) [b]				x
		Green/black tea (g/day) [b]				x
		Iron (mg/day) [b]				x
		Magnesium (mg/day) [b]				x
		MUFA (g/day) [b]				x
		Niacin (mg/day) [b]				x
	Dietary and	**n-3 PUFA** (g/day) [b]				x

	nutritional intake	**n-6 PUFA** (g/day) [b]				x
		Onion (g/day) [b]				x
		Protein (g/day) [b]				x
		PUFA (g/day) [b]				x
		Riboflavin (mg/day) [b]				x
		SFA (g/day) [b]				x
		Selenium (mg/day) [b]				x
		Thiamin (mg/day) [b]				x
		TFA (g/day) [b]				x
		Vitamin A (RE/day) [b]				x
		Vitamin C (mg/day) [b]				x
		Vitamin D (μg/day) [b]				x
		Vitamin E (mg/day) [b]				x
		Zinc (mg/day) [b]				x
Confounders	**Others covariates**	**Sex** ("boy" or "girl")	x	x	x	x
		Age (years)	x	x	x	x
		Pubertal stage - Tanner A and B ("2", "3", "4", or "5")	x	x	x	x
		Body mass index (kg/m^2, Cole's categories)	x	x	x	x
		Socioeconomic status (0-9 points)	x	x	x	x
		Moderate-to-vigorous physical activity (min/day)	x	x	x	x
		Sedentary behaviour (min/day)	x	x	x	x
		Smoking habits ("non-smoker", "former smoker", "occasional smoker", or "current smoker")	x	x	x	x

Abbreviations: CRP, C reactive protein; IL-6, Interleukin 6; C3, Complement component 3; C4, Complement component 4; SFA, Saturated fatty acid; MUFA, Monounsaturated fatty acid; PUFA, Polyunsaturated fatty acid, n-6, Omega 6; n-3, Omega 3; AA, Araquidonic acid; LA, linoneic acid; ALA, Alpha-linolenic acid; EPA, Eicosapentaenoic acid; DHA, Docosahexaenoic acid; TFA, Trans fatty acid; %en, energy intake percentage; DII, Dietary inflammatory index.

[a] This table shows each variables considered and between brackets are the measurement units and/or description of categories.

[b] These variables were considered to calculate DII score.

[c] This variable was considered as confounder in fully adjusted models (Paper II, III, and IV).

[d] This variable was considered as confounder in fruit, vegetable soup and vegetable variety fully adjusted models.

[e] This variable was considered as confounder in vegetable, vegetable soup and fruit variety fully adjusted models.

[f] This variable was considered as confounder in vegetable and fruit fully adjusted models.

The final median values for each category (higher/lower) were 0.11/0.92 mg/L for CRP, 1.90/4.20 ng/L for IL-6, 107.00/127.00 mg/dL for C3, and 17.00/25.55 mg/dL for C4.

We have also calculated an overall inflammatory biomarker score, assigning one point to subjects who were above the sex- and age-adjusted medians and zero to those who were below, for each inflammatory biomarker and totaled all points assigned. The overall inflammatory biomarker score varies from zero to four points. Two categories were defined: 0–1 (49.9%) and 2–4 (50.1%) inflammatory biomarkers above the median.

6.2.2. Dietary intake assessment

A self-administered, semi-quantitative food-frequency questionnaire validated for a Portuguese population[328] and adapted to adolescents[329] was used to assess dietary intake in the previous 12 months. The food-frequency questionnaire lists 91 food and beverage items or categories with a standard portion size, nine response options ("from never or less than once per month" to "six or more times per day"), and a seasonal alternative. Blank lines were included for participants to add any food that was not listed. The portion size in grams was multiplied by the multiple/fraction of daily frequency intake and by a seasonality variation factor for each option selected, and dietary intake was estimated. The Food Processor Plus program version SQL (ESHA Research, Salem, OR, USA), supplemented with the Portuguese food-composition databases,[330, 331] was used to convert food to energy and nutrient intakes.

We included only accurate food-frequency questionnaires, using the Goldberg cut-off method[332] adapted by Black,[333] for the determination and exclusion of dietary assessment misreporting. Thus, we calculated the basal metabolic rate using the Schofield equation,[334] considering sex and age, and a ratio energy intake/basal metabolic rate to compare with 95% confidence limits (cut-offs). The cut-offs for our sample were determined using the following: mean physical activity level, number of days of dietary assessment, within-subject coefficient of variation in energy intake, between-subject variation in physical activity, and variation in basal metabolic rate. The mean physical activity level was 1.23, calculated using accelerometer data (counts/minutes and daily use time) per the Trost equation.[335] We considered 21 days of dietary assessment, according to Black, for the food-frequency questionnaire.[333] The within-subject coefficient of variation in energy intake was calculated considering mean and standard deviation of energy intake in our sample and the number of dietary assessments. The between-subject variation in physical activity was calculated considering the mean and standard deviation of physical activity level in our sample. A figure of 8.5% was used for the coefficient of variation of repeated basal metabolic rate measurements, as Black suggested.[333] The cut-offs achieved were 0.61 and 2.48; therefore, adolescents with energy intake/basal metabolic rate outside of this

range were considered to have misreported dietary assessment, and 150 adolescents were excluded from the statistical analysis.

6.2.2.1. Nutrients intake

The nutrients intake considered were protein, total carbohydrates, sugars, dietary fiber, total fat, saturated fat acids (SFA), monounsaturated fat acids (MUFA), polyunsaturated fat acids (PUFA), trans fat acids (TFA), and alcohol. In relation to PUFA, we also determined total intake of n-6 PUFA, and following fatty acids: araquidonic acid (AA), linoneic acid (LA); and total intake of n-3 PUFA and following fatty acids: alpha-linolenic acid (ALA), eicosapentaenoic acid plus docosahexaenoic acid (EPA+DHA). The nutrients contribution of total energy intake (%en) was calculated considering 4kcal/g for protein, total carbohydrates and sugar, 9kcal/g for fat and 7kcal/g for alcohol. For dietary fiber intake, results were expressed as g/1000kcal.

6.2.2.2. Fruit, vegetable, vegetable soup portions and the '5-a-day' recommendation

To quantify the number of portion from food categories (fruits, vegetables and vegetable soup), we have summed the daily intake in grams of all items from each food group, and divided them by the mean portion size (160g for fruit or vegetable, 80g for green pulses), according to Food Guide for the Portuguese Population.[75]

For the fruit category, we have included apple/pear, orange/tangerine, banana, kiwi, strawberry, cherry, peach/plum, melon/watermelon, persimmon, fig/medlar/apricot, grape and papaya/mango; and excluded canned fruit in syrup and fruit juices because their sugar content is unknown.

For the vegetable category, we have considered white/savoy cabbages, bunch/portuguese cabbages, curly kale, broccoli, cauliflower/brussel sprout, turnip sprout/turnip greens/spinach, green been, lettuce/watercress, onion, carrot, turnip white, tomato, pepper, cucumber, as well green pulses in item peas/broad beans; and excluded potatoes and dried pulses because their starch content is very much higher than others foods in this group and because there is no consensus about inclusion of those foods in the vegetable category.[336]

For the vegetable soup, we have considered a portion size of 295g, and it was treated as a different food category because it is a single item from food-frequency questionnaire. Vegetable soup is considered an important source of vegetable intake in Portuguese diet, being consumed mostly as a starter of the main meals (lunch and dinner), and recommendation of two portions/day is usually recognized as an important Portuguese healthy dietary guideline.

For the 5-a-day recommendation, we have totaled the number of portions of each food categories (fruit, vegetable and vegetable soup) to analyze adherence to the 5-a-day recommendation. The 5-a-day is a public health message and it is widespread and used in several countries to encourage individuals to achieve the goal of eating five or more daily servings of fruit and vegetable.[337]

Participants were grouped according to daily portion intake, into low or adequate, of fruits (<3 or ≥3 portions/day), vegetables (<3 or ≥3 portions/day), and vegetable soup (<2 or ≥2 portions/day), in accordance with Portuguese guidelines.[75] In addition, to analyze adherence to the 5-a-day recommendation, participants were grouped the in very low (<1 portion/day), low (≥1 to <5 portions/day), or adequate (≥5 portions/day) fruit and vegetable consumptions.

6.2.2.3. Fruit and vegetable variety

The variety of intake of fruits and vegetables was defined by scores considering the total number of unique individual/categories of fruits or vegetables consumed at least once per month over the past 12 months; no point was attributed for consumption "< once per month", while 1 point was given for an intake of "≥1 time per month".

For the fruit variety, the score considered a maximum of 12 categories, as follows: apple/pear, orange/tangerine, banana, kiwi, strawberry, cherry, peach/plum, melon/watermelon, persimmon, fig/medlar/apricot, grape, and papaya/mango; it excluded candied fruit and fruit juices because their sugar content is unknown and also to avoid replicating the same fruit variety listed above.

For the vegetable variety, the score considered a maximum of 15 categories, as follows: white/savoy cabbages, bunch/Portuguese cabbages, curly kale, broccoli,

cauliflower/Brussel sprout, turnip sprout/turnip greens/spinach, green bean, lettuce/watercress, onion, carrot, turnip, tomato, pepper, cucumber, pea/broad bean; it excluded starchy vegetables like potatoes and dried pulses because their starch content is very different from other foods and because there is no consensus about the inclusion of these foods in the vegetable category.[336, 338]

Adolescents were classified as having low-, medium-, and high-variety intake, according to the tertiles of the fruit and vegetable variety scores, considering the following: 1st tertile ≤ 6 categories/month, 2nd tertile 7–12 categories/month, and 3rd tertile ≥ 13 categories/month for vegetable variety; and for fruit variety, 1st tertile ≤ 9 categories/month, 2nd tertile 10–11 categories/month, and 3rd tertile = 12 categories/month.

6.2.2.4. Dietary inflammatory index

As explained above (in 4.2.4.3 Dietary and nutritional intake, na página 51), the DII is a literature-based tool[299] that measures the diet' inflammatory properties giving a score to each individual. The DII score points 45 food parameters (food parameter-specific overall inflammatory effect score) according to their inflammatory effects to achieve to a final score ranging from -8.87 to 7.98. This score is interpreted as strongly anti-inflammatory (lower values) to strongly pro-inflammatory (higher values).

In this study, the DII score was calculated considering 31 food parameters (available on Table 2, page 73). Eugenol, garlic, ginger, saffron, turmeric, flavan-3-ol, flavones, flavonols, flavonones, anthocyanidis, isoflavones, pepper, thyme/oregano, rosemary were not included because no information was available for these components in the Food Processor nutritional database neither those herbs or spices were included in the food-frequency questionnaire.

We calculated the DII score according described by Shivappa.[299] Briefly describing our calculation, first a mean and standard deviation were calculated for the 31 food parameters available because no global data for adolescent is available. Second, a z-score of each food parameter and for each participant was calculated. Third, each individual z-score was converted to a centred percentile. Fourth, each centred percentile was multiplied by its respective food parameter-

specific overall inflammatory effect score, published by Shivappa[299], and then a food parameter-specific DII score is obtained. Finally, the 31 food parameter-specific DII score was totaled and an individual DII score was obtained.

Our DII score values were ranging from -5.36 to 4.24, and it was categorized, based on tertiles values, in accordance with Shivappa,[302] considering low (1st tertile: <-1.34), medium (2nd tertile: -1.34 to 1.41), and high (3rd tertile: >1.41) pro-inflammatory dietary property.

6.2.3. Others covariates assessment

Other important key variables should be included in statistical analysis and it is a recommended practice,[70, 100] in order to control their effect on inflammatory biomarkers. So we considered the followed variables, recognized as important inflammatory biomarkers modifiers:[69, 70, 100] sex, age, physical activity, sedentary time, body mass index, and smoking habits. It seems important to include measure of pubertal stage, considering that we are working with adolescents, and also the socioeconomic status.

6.2.3.1. Physical activity and sedentary time assessment

GT1M Actigraph accelerometers (ActiGraph, Pensacola, Florida, USA) were used to assess physical activity and sedentary time. This is a lightweight, biaxial monitor that adolescents wore attached tightly at the hip on the right side of the body with the notch facing upwards. It was used during all waking hours and removed during water-based activities for five consecutive days (three weekdays and two weekend days). The epoch length was set to 2 seconds to allow a more detailed estimate of physical activity intensity.

An automated data-reduction program (ActivLive 6.12, ActiGraph, Pensacola, Florida, USA) was utilized to analyze accelerometer data from individual participants. Non-wearing time was considered when 60 minutes of consecutive zeros were flagged. A valid day was considered to consist of at least 8 hours of accelerometer use. The participants had to have at least three valid days to be included (two weekdays and one weekend day). This combination of hours and days was studied to achieve a reliability of 90%.[339]

After screening, the cut-points proposed by Evensons et al[340] were used to determine physical activity intensities according to the raw activity counts. Physical activity was expressed in mean counts/min and also in estimates of the time spent in moderate-to-vigorous physical activity and sedentary time, using minutes/day.

6.2.3.2. Anthropometric data

Weight and height were measured with a scale (TANITA Inner Scan BC532, Tokyo, Japan) and a stadiometer (SECA 213, Hamburg, Germany) respectively, with the adolescent standing upright, lightly dressed, and without shoes. We calculated the body mass index from the ratio of weight (kg) to height squared (m^2), and adolescents were classified according to Cole's body mass index categories into underweight, normal weight, overweight and obese.[341]

6.2.3.3. Pubertal stage

Adolescents self-reported their pubertal stage relatively to secondary sex characteristics, according to the criteria of Tanner and Whitehouse.[39] The pubertal development is assigned on a scale from 1 (prepubertal) to 5 (adult). There are two different charts, Tanner A and B, by sex. Tanner A indicates the stage of breast development in girls and genitalia development (penis size and testicular volume) in boys; and Tanner B indicates the stage of public hair distribution in both sexes.[41] This assessment based on Tanner's charts can be performed by a physician or to be self assessed, maintaining the privacy of the adolescent in the second option.[342]

6.2.3.4. Socioeconomic status

The family affluence scale was used to assess socioeconomic status of adolescents.[53] This is a self-filled questionnaire considering things about adolescents' family as such number of car, bedrooms, vacations, computers, and other. This scale was developed specifically to measure children and adolescents socioeconomic status in the context of the Health Behaviour in School-Aged Children Study and ranks the participants from 0 to 9 (from lower to highest socioeconomic status).[53]

6.2.3.5. Smoking habits

Adolescents self-reported their smoking habits and were classified according to the World Health Organization's criteria[343] as: non-smokers, former smokers (individuals who had stopped smoking for at least 6 months), occasional smokers (individuals who smoked, on average, less than one cigarette a day), and current smokers (individuals who smoked at least one cigarette a day).

6.3. Statistical Analyses

The statistical analysis was performed, as described in Table 3, using the statistical package SPSS®, version 21.0 (SPSS Inc., Chicago, IL, USA), considering a 0.05 level of significance and 95% CI (confidence interval).

Participants' characteristics are presented in global and by sex. Qui-square test for categorical variables, Student T test for continuous normally distributed variables, and Mann-Whitney U test for skewed variables were used to assess differences between variables. Spearman's test was used to verify the correlation between dietary components and dietary inflammatory index (DII).

Table 3: Statistical analysis methods considered for this thesis

Statistical methods		Papers			
		I	II	III	IV
Descriptive	Percentages	x	x	x	x
	Median	x	x	x	x
	Interquartile range	x	x	x	x
	Mean				x
	Standard deviation				x
Associations	Mann-Whitney U test	x	x	x	x
	Student T test	x			
	Qui-square test	x	x	x	x
	Spearman's correlation tests				x
Models	Binary logistic regression	x	x	x	x

To estimate the magnitude of association between dietary and nutritional intake variables and inflammatory biomarkers we have constructed crude and adjusted binary logistic regression models considering odds ratio (OR), 95% confidence intervals (95%CI), and p values for trend (p_{trend}). Categories of each inflammation biomarker and the overall inflammation biomarkers score were

considered, as dependent variables, dietary and nutritional intake variables, as predictors, and other variables, as confounders. For the fully adjusted models, confounders are summarized in Table 2.

Dietary and nutritional intake variables included as predictors in the models were as following:

- **Paper I:** tertiles of SFA, MUFA, AA, LA, ALA, EPA+DHA, and TFA, using the first tertiles as reference, that entered into regression models together;
- **Paper II:** categories of fruit, vegetable, vegetable soup portions and the 5-a-day recommendation, using the major portion as reference, in separated regression models;
- **Paper III:** tertiles of fruit variety and vegetable variety, using the first tertiles as reference, in separated regression models;
- **Paper IV:** DII score in tertiles, using the first tertiles as reference.

To calculate the power of each model was used G*power software, version 3.1 (Faul, Erdfelder, Lang, & Buchner, 2007). *Post hoc* for logistic regression was performed for each statistically significant model, considering the sample size and odds ratio of the model, a null hypothesis value of 0.5, and two-tailed significance of 5%, achieving statistical powers higher than 0.80 for all models.

7. Papers

7.1. PAPER I

Fatty-acids intake and low-grade inflammation in Portuguese adolescents: the LabMed physical activity study.

[Submitted for publication]

Fatty-acids intake and low-grade inflammation in Portuguese adolescents: the LabMed Physical Activity Study

Juliana Almeida-de-Souza[1,2,*], Rute Santos[3,4,5], Luís Lopes[3], Sandra Abreu[3], Carla Moreira [3], Jorge Mota[3], Pedro Moreira[2,3,6].

[1] School of Health, Polytechnic Institute of Bragança; Avenida D. Afonso V - 5300-121 Bragança, Portugal.

[2] Faculty of Nutrition and Food Sciences, University of Porto, Rua Dr. Roberto Frias 4200-465 Porto, Portugal.

[3] Research Centre in Physical Activity, Health and Leisure, Faculty of Sport, University of Porto, Rua Dr. Plácido Costa n.91 4200-450 Porto, Portugal.

[4] University Institute of Maia; Avenida Carlos de Oliveira Campos - Castêlo da Maia 4475-690 Maia, Portugal.

[5] Early Start Research Institute, University of Wollongong; Wollongong NSW 2522, Australia.

[6] Institute of Public Health, University of Porto; Rua das Taipas nº135 4050-600 Porto, Portugal.

* Correspondent author: julianaalmeida@ipb.pt; Telephone number: +351-96-438-6309; Fax number: +351-273-327-915.

Short Title: Fatty acids and inflammation in adolescents

Abstract: Low-grade inflammation is considered an important factor in chronic disease development and may be influenced by diet. We performed a cross-sectional study in 412 adolescents (14.9±1.72 years), mostly girls (52.4%), to study the association between fatty-acid intake and low-grade inflammation. We assessed fatty acids in percentage of energy intake (%en), determined by a food-frequency questionnaire, and then we categorized them into tertiles. We collected blood samples to assess C-reactive protein, interleukin-6, and complement components 3, and 4, and create categories of lower or higher (inflammatory state) for each biomarker, considering sex- and age-adjusted median values. An overall inflammatory biomarker score was computed by adding the biomarkers in the categories (it was assigned one point if it was higher or zero if it was lower), and classified as 0–1 or 2–4 biomarkers above the median (lower or higher inflammatory state). Odds ratio (OR) and 95%

interval confidence (95%IC) were calculated by binary logistic regression, adjusted for confounders. Adolescents with a higher consumption of saturated fatty acids (SFA) (≥11.12%en), compared with lower (≤9.72%en), had higher odds of having a higher interleukin-6 (OR=2.16, 95%IC:1.02-4.56, p_{trend}=0.05), and the inflammatory score (OR=2.51, 95%IC:1.20-5.27, p_{trend}<0.05). Higher intakes of α-linolenic acid (ALA) (≥0.50%en) and eicosapentaenoic plus docosahexaenoic acids (EPA+DHA) (≥0.22%en), compared with lower (≤0.42%en and 0.12%en, respectively), had lower odds of having a higher inflammatory score (OR=0.49, 95%CI:0.26-0.95 and OR=0.46, 95%CI:0.23-0.93, respectively, p_{trend}<0.05 for both). Greater intakes of ALA and EPA+DHA and a smaller intake of SFA can be protective for low-grade inflammation in adolescents.

Keywords: Saturated fatty acids (SFA); n-3 PUFA, ALA; EPA; DHA; C-reactive protein (CRP); interleukin-6 (IL-6); complement component 3 (C3); complement component 4 (C4); and inflammatory biomarker score.

Introduction

Inflammation is an essential component of immunity and acts as a host defence.[1] Acute inflammation is a physiological response to injury, and so when it is controlled, it is very important to health. However, in chronic inflammation, the regulation is lost and it can become a pathological process.[2]

Low-grade inflammation is characterized by raised blood concentrations of inflammatory biomarkers,[3] such as acute phase proteins and cytokines,[2] and this increase in systemic inflammatory biomarkers can be minimal or absent, unlike what happens with chronic or acute inflammation.[2]

In adults, low-grade inflammation has been correlated with a set of chronic conditions,[1; 2] such as obesity,[4] diabetes,[4; 5] cardiovascular diseases,[6; 7] and cancer.[8; 9] This association has also been found in the paediatric age for obesity,[10; 11] metabolic syndrome,[12; 13] and atherosclerosis.[14]

Nutrition is an important regulatory factor in immune response, because an optimal nutrition is required for a healthy immune balance: if, on the one hand, malnutrition leads to immunosuppression and, on the other hand, overnutrition increases the susceptibility to an inflammatory condition.[15] Many foods, nutrients, and non-nutrient food components modulate inflammation.[1] Calder et al.[3] reviewed several nutritional factors and identified the most relevant and major contributors to inflammation: polyunsaturated fatty acids (PUFA), especially omega-3 (n-3) PUFA, vitamin C, vitamin E, and carotenoids as protective nutrients; alcohol intake works in a U-shape for low-grade inflammation, and one or two daily alcoholic beverages as more protective; and saturated fatty acids (SFA) and trans fatty acids (TFA), as promoters of inflammation. The results of this review were based almost entirely on studies based on adult samples, since studies with children and adolescents are scarce, and, therefore, not much is known about the relationship between nutrient intake and low-grade inflammation in adolescents. In fact, the study of the associations between dietary intake and inflammatory biomarkers in healthy young populations is recognized as challenging.[16]

The early identification of a biomarker with diagnostic value to prevent future damage or facilitate early treatment is desirable;[17] however, the best biomarker or set of biomarkers that can fully describe the low-grade inflammation state is not yet

78 defined.[1; 2; 3] Therefore, approaches that use a cluster of biomarkers to describe the
79 inflammatory state seem sensible.[1]

80 In this context, we aimed to study the main dietary fatty acids as predictors of
81 low-grade inflammation, measured by a set of biomarkers and an overall inflammatory
82 biomarker score, in apparently healthy adolescents.

83 **Materials and Methods**

84 *Subjects and Study Design*

85 The present study uses baseline data from a school-based prospective cohort study
86 carried out in five schools in the north of Portugal (2011), that have collaboration
87 agreements previously established with our research Centre. This is the Longitudinal
88 Analysis of Biomarkers and Environmental Determinants of Physical Activity Study
89 (LabMed Physical Activity Study), which the main aim is evaluate the associations of
90 dietary intake and fitness levels on blood pressure levels of adolescents. The full
91 description of this study is reported elsewhere.[18; 19]

92 Briefly, the Sample size was previously estimated in 1086 subjects, considering a
93 prevalence of 14% to the combined healthy diet/physical activity pattern exposure,[40]
94 for have a power of 0.80, a 2-tailed significance of 5%, and an expected dropout rate of
95 20%. So, all students from 7th and 10th grade were invited (N=1678), and who
96 accepted entry in the study (n=1229), although only the subjects that had absence of
97 known disease undergone blood sampling. The measurements were conducted at
98 schools.

99 For this cross-sectional, the sample comprised 412 healthy adolescents (12–18 years
100 old), most girls (n=216), which simultaneously accepted to undergo blood sampling
101 (n=534), and fulfilled an accurate (misreporting excluded, as described in 2.4. Dietary
102 intake assessment) food-frequency questionnaire (n=622).

103 *Ethical issue*

104 The LabMed Physical Activity Study were performed in accordance with the World
105 Medical Association's Helsinki Declaration for Human Studies, participants and their
106 parents/guardians provided written informed consent. Also, ethical approval were
107 obtained by the Portuguese Data Protection Authority (#1112434/2011), the

Portuguese Ministry of Science and Education (0246200001/2011), and the Faculty of Sport, University of Porto.

Inflammatory biomarkers assessment and inflammatory biomarkers score

Blood samples collection was performed after a minimal of ten hours of fasting, refrigerated (4º-8°C), and sent to laboratory to determine the inflammatory biomarkers. High sensitive C-reactive protein (CRP) was assessed by the Latex-enhanced turbidimetric assay (Siemens Advia 1600/1800, Erlangen, Germany); Interleukin-6 (IL-6), by the Chemiluminescence immunoassay (Immulite 2000, Diagnostic Products Corporation, Los Angeles, CA) to determine); and Complement component 3 (C3) and 4 (C4), by the Immunoturbidimetric assay (Siemens Advia 1600/1800, Erlangen, German).

Considering that the increase of inflammatory biomarkers in low-grade inflammation can be minimal or absent,[2] and the lacking of accepted international cut-points for adolescents, participants were grouped in two categories based on median values adjusted for age and sex: lower or higher (inflammatory state). The final medians of each category (lower/higher) were 0.11/0.92 mg/L for CRP, 1.90/4.20 ng/L for IL-6, 107.00/127.00 mg/dL for C3, and 17.00/25.55 mg/dL for C4.

We calculated an overall inflammatory biomarker score assigning one point to subjects who were above the sex-age-adjusted medians or zero for subjects who were below, for each inflammatory biomarker, and summing all points assigned. The overall inflammatory biomarker score varies from zero to four points. Two categories were defined: 0-1 (49.9%) or 2-4 (50.1%) inflammatory biomarkers above the median.

Dietary intake assessment

Dietary intake was assessed by validated self-administered semi-quantitative food-frequency questionnaire for Portuguese population,[20] and adapted to adolescents.[21] It measures the food consumption in previous 12 months by a list of 91 food and beverage items or categories, with nine frequency possibilities (from “never or less than once per month” to “six or more times per day”), with a standard portion sizes, and with a seasonality option. There is still space available for inclusion of any food not listed. Dietary intake quantification was made multiplying the portion size in

grams by the multiple/fraction of daily frequency intake, and by a seasonality variation factor. Food to energy and nutrients intake conversion was performed using The Food Processor Plus program version SQL (ESHA Research, Salem, OR, USA). This database was supplemented with the Portuguese food composition databases.[22; 23]

We excluded food-frequencies questionnaire with implausible energy intake reported by determination of dietary assessment misreporting, using the Goldberg' cut-off method,[24] adapted by Black.[25] Briefly, the Schofield equations[26] were used to estimate the basal metabolic rate. A ratio energy intake/basal metabolic rate was compared with 95% confidence limits (cut-offs). The cut-offs were calculated using five parameters: 1) the sample mean physical activity level, calculated from accelerometers data (as explained forward), using counts/minutes and daily use time, as Trost equation,[27] reaching a value of 1.23; 2) the days of diet assessment was considered in 21, as Black recommendation for food-frequency questionnaire;[25] 3) the within-subject coefficient of variation in energy intake, calculated considering mean, standard deviation of energy intake in our sample and number of diet assessment; 4) the between-subject variation in physical activity, calculated considering mean and standard deviation of physical activity level in our sample, avoiding the use of theoretical values; 5) the coefficient of variation of repeated basal metabolic rate measurements, considered a figure of 8.5%, as Black indication.[25] The cut-offs achieved were 0.61 and 2.48; then, adolescents with energy intake/basal metabolic rate below 0.61 and over 2.48 were excluded from statistical analysis (n=150), by reason of dietary intake misreporting.

The nutrients intake considered were protein, total carbohydrates, sugars, dietary fiber, total fat, saturated fat acids (SFA), monounsaturated fat acids (MUFA), polyunsaturated fat acids (PUFA), trans fat acids (TFA), and alcohol. In relation to PUFA, we also determined total intake of n-6 PUFA, and following fatty acids: araquidonic acid (AA), linoneic acid (LA); and total intake of n-3 PUFA and following fatty acids: alpha-linolenic acid (ALA), eicosapentaenoic acid plus docosahexaenoic acid (EPA+DHA). The nutrients contribution of total energy intake (%en) was calculated considering 4kcal/g for protein, total carbohydrates and sugar, 9kcal/g for fat and 7kcal/g for alcohol. For dietary fiber intake, results were expressed as g/1000kcal.

Covariates

Other important key variables should be included in our models and it is a recommended practice,[1; 2] in order to control their effect on inflammatory biomarkers. So we considered the followed variables, recognized as important inflammatory biomarkers modifiers:[1; 2; 3] sex, age, physical activity, sedentary time, body mass index, and smoking habits. It seems important to include measure of pubertal stage, once we are working with adolescents, and also the socio-economic status, because social disadvantage have been associated with increased inflammation in adolescence.[28; 29; 30]

Physical activity and sedentary time assessment: To measure physical activity and sedentary time, we used accelerometer's data from the GT1M Actigraph (ActiGraph, Pensacola, Florida, USA). This is a lightweight and biaxial monitor that participants wore tightly attached to the waist (on the right side of hip), over five consecutive days (three weekdays and two weekend days), during waking hours and removed it during sleep and water-based activities. The epoch length was set to 2 seconds to allow a more detailed estimate of physical activity intensity.

We work with an automated data reduction program (ActivLive 6.12, ActiGraph, Pensacola, Florida, USA) to treat accelerometer data. Each 60 minutes of consecutive zeros were detected as non-wear time and at least 8 hours of data were considered as a valid day. To be included, adolescents had to have at least two weekdays and one weekend valid day. This combination of hours and days were studied to achieve a reliability of 90%.[31]

The time spent in different physical activity intensities and sedentary time was determined by the raw activity "counts", considering Evenson's et al cut-points.[32] Moderate-to-vigorous physical activity and sedentary time were expressed as average minutes per day.

Anthropometric assessment data: Height and weight were assessed with a portable stadiometer (SECA 213, Hamburg, Germany) and an electronic scale (TANITA Inner Scan BC532, Tokyo, Japan), respectively, according to standard procedures.[33] Body mass index was calculated from the ratio of weight (kg) to height squared (m^2) to classified participants according to Cole's cut-offs.[34]

Pubertal stage: Self-assessment of pubertal stage, from 1 to 5, relatively the secondary sex characteristics was performed, according to the criteria of Tanner and Whitehouse.[35] Tanner A measured the stages of breast (in girls) and genitalia (in boys) development; and Tanner B measures the stages of public hair distribution in both sexes.

Socio-economic status: Adolescents self-reported their socio-economic status with the Family Affluence Scale,[36]. This scale ranks adolescents' socio-economic status from 0 to 9, considering higher scores as higher socioeconomic status.

Smoking habits: Smoking habits were self-reported and coded according to the World Health Organization criteria: non-smokers, former smokers, occasional smokers, and current smokers. [37]

Statistical Analyses

Participants' characteristics are presented as percentages for categorical variables, and as means and standard deviation for continuous variables. To assess differences between sexes, we performed Qui-square test (for categorical variables), Student T test (for continuous normally distributed variables) and Mann-Whitney U test (for continuous non-normally distributed variables). We also used Qui-square test to assess differences between inflammatory biomarkers categories and nutrient intakes tertiles in percentage of energy or g/1000kcal (for dietary fiber).

Binary logistic regression models were constructed to estimate the magnitude of association between fatty acids intake and low-grade inflammation, by odds ratio (OR) and 95% confidence interval (CI) calculation. We considered inflammatory biomarkers and the overall inflammatory biomarkers score as dependent variables; fatty acids (SFA, MUFA, AA, LA, ALA, EPA+DHA, and TFA) as predictors, that entered in the regression models together. We performed a crude models, sex-adjusted models, and fully adjusted models, that were adjusted for sex, age, pubertal stage (Tanner A and B), body mass index, socio-economic status, sedentary time, moderate-to-vigorous physical activity, smoking habits.

Post hoc power calculations were performed considering our sample size, our range of odds ratio, a null hypothesis value of 0.5, and 5% of significance, achieving powers of ≥0.88 for all statistically significant models.

A 0.05 level of significance and 95% CI were considered. Data analysis was performed using the statistical package SPSS®, version 21.0 (SPSS Inc., Chicago, IL, USA) and G*power, version 3.1 (Faul, Erdfelder, Lang, & Buchner, 2007), were used to power calculation.

Results

The sample characteristics are presented in table 1. Girls presented on average a lower CRP, less alcohol intake, less moderate-to-vigorous physical activity, lower energy intake and a higher sedentary time than boys ($p<0.05$ for all).

As depicted in table 2, adolescents that consumed less ALA (≤0.42%en) had higher prevalence of have a higher overall inflammatory biomarker score ($p<0.05$), this is 2 to 4 inflammatory biomarkers above the median.

As it can be observed in table 3, adolescents with higher intake of SFA (≥11.12%en), when compared with a lower intake (≤9.72%en), had a higher odds of having a higher IL-6 in all models, p for trend <0.05 for crude and sex-adjusted model, however fully adjusted was marginally significant for trend (OR=2.16, 95%CI:1.02-4.56, p_{trend}=0.051). The higher intake of SFA was also inversely associated with the overall inflammatory biomarker score (OR=2.51, 95%CI:1.20-5.27, p_{trend}=0.015), in fully adjusted model. In addition, adolescents with higher consumption of ALA (≥0.50%en) and EPA+DHA (≥0.22%en), than adolescents with lower intake (≥0.42%en and ≥0.12%en, respectively), had a lower odds of having high overall inflammatory biomarkers score (OR=0.49, 95%CI:0.26-0.95, and OR=0.46, 95%CI:0.23-0.93, respectively), p for trend <0.05 for both in fully adjusted models.

Discussion

The main finding of this study suggests that the fatty acids that have greater influence on low-grade inflammation in adolescents are SFA, acting as pro-inflammatory dietary factors, and ALA, EPA, and DHA, acting as anti-inflammatory dietary factors. Intake of SFA had an impact on IL-6 and the overall inflammatory biomarker score, while intake of ALA, EPA, and DHA have showed an influence on the overall inflammatory biomarkers score (based on four inflammatory biomarkers). These results support the recommendations of consuming n-3 PUFA, especially for ALA, EPA, and DHA, and of limiting the SFA intake as important factors for the prevention of a low-grade

inflammation state. To the best of our knowledge, no prior study has specifically analysed the main fatty acids predictors, namely AA, LA, ALA, EPA and DHA, of low-grade inflammation in apparently healthy adolescents.

We showed that SFA daily intake higher than 11.1% of energy intake significantly increased in more than two times the odds of having a higher IL-6 or two to four inflammatory biomarkers above the median (our overall inflammatory biomarker score in categories). In accordance with our results, Kalogeropoulos et al.[38] have reported that IL-6 had positive sex-adjusted correlation with dietary SFA and was predicted by a higher concentration of plasma SFA, after adjustment for confounders, in a cross-sectional study in healthy adult Greeks. However, Aeberli et al.,[39] in a cross-sectional study conducted in Swiss children and adolescents ages between 6 to 14 years old, found no association between SFA and IL-6, but did CRP, contrary to our findings. We have showed no association between SFA and CRP. In accordance with our results, Santos et al.[40] reviewed evidence regarding the association of SFA with inflammatory biomarkers and showed that most studies (3 in 5) reported no significant associations.

For n-3 PUFA, we have also shown that ALA and EPA+DHA daily intakes of 0.50% and 0.22% of energy intake or more, respectively, significantly reduced about half the odds of having two to four inflammatory biomarkers above the median (our overall inflammatory biomarker score in categories). Other authors have also found an influence of n-3 PUFA on inflammatory biomarkers in adult-based samples. Poudel-Tandukar et al.[41] showed that dietary intake of ALA was inversely associated with CRP levels among Japanese men and Navarro et al.[42] and Kubota et al.[43] showed that EPA and DHA intake was associated with decreased CRP levels in Americans and Japanese, respectively. In contrast, Aeberli et al.[39] reported a positive relationship between PUFA (no distinction between fatty acids, so n-3 and n-6 PUFA were treated together) and CRP in children and adolescents. However, the roles of n-3 and n-6 on inflammation are different. In fact, Calder et al.[44] reviewed the role of marine n-3 PUFA on inflammatory processes and concluded that EPA and DHA are able to partly inhibit a number of aspects of inflammation, including production of eicosanoids from the n-6 PUFA. The role of to ALA in reducing low-grade inflammation has been attributed to the potential of ALA to be a substrate for the synthesis of EPA and DHA

296 and so to increase the serum concentrations of EPA or EPA+DPA and the content of
297 EPA in mononuclear cells, such as neutrophils and monocytes.[45]

298 In addition, the recommended daily intake of EPA+DHA fatty acids for adults, found
299 especially in fish and fish oil, varies from 250 to 500mg/day, or up to 0.06% to 0.12%
300 of energy intake.[46] Our results suggest that, in order to prevent low-grade
301 inflammation, adolescents may require a higher intake of these fatty acids (≥0.22%en);
302 nevertheless, our cross-sectional design precludes us from establishing a dose-response
303 relationship. Indeed, the study of dose-response relationships between EPA+DHA and
304 inflammation is timely, and some have suggested that a dose of at least 2g/day, a value
305 that is also above the recommended, might be needed to prevent low-grade
306 inflammation.[44] Regarding ALA, which is found especially in seeds, nuts, vegetable
307 oils, and green leaves, the cut-off in our study (≥0.5%en) coincided with the minimal
308 recommended range (varying from 0.5% to 2%, between several organizations, or
309 1.1-1.6g/day),[46] so an intake in accordance with the recommendations seems to be
310 protective against low-grade inflammation. However, no consensus is found in the
311 quantities of ALA necessary to reduce inflammatory biomarkers, but some authors[47;
312 48] referred to a quantity above the recommendation. Review studies about
313 dose-response relationships have indicated that, on the one hand, a minimum of
314 14g/day[47] is necessary to reduce inflammatory biomarkers, and, on the another hand, a
315 quantity of 4.5g/day[48] is sufficient. Considering intervention studies, a recent
316 randomized study in obese adult Chinese showed that 4.0g of ALA daily for 12 weeks
317 decreased serum concentration of IL-6 and tumour necrosis factor-α, after weight
318 reduction, compared with the before-ALA treatment in the intervention group and the
319 after-placebo treatment in the control group.[49]

320 Interestingly, our inflammatory biomarker score was stronger in showing the
321 relationship between fat intakes than a single biomarker, in the fully adjusted model. In
322 fact, the inflammatory biomarkers in general are considered non-specific
323 pro-inflammatory response markers in healthy people,[1] and our overall inflammatory
324 biomarker score was shown to be a more complex and integrated assessment of
325 low-grade inflammation rather than just an inflammatory biomarker alone. This score
326 considers who is above the median adjusted for age and sex, taking into account the
327 effects of all inflammatory biomarkers, those that were shown to have a relationship
328 with fat intake (as IL-6 for SFA) and those that did not (as CRP, C3, and C4), and it

seems to represent better low-grade inflammation in this group of adolescents. With this inflammatory score, there was more possibility to overcome the limitation to study the associations between dietary intake and inflammatory biomarkers in a healthy young population, than was previously recognized.[16] Other authors have used other inflammatory scores, with different biomarkers, as in the finding of van Bussel et al.[50] that some healthy diet parameters were associated with less low-grade inflammation. This finding was measured by an inflammatory biomarker score in a longitudinal study in Dutch adults. To the best of our knowledge, no other prior study has shown a relationship between fatty acids and an inflammatory score.

The strengths of this study include, beyond the novelty of its aim, the use of objectively measured physical activity,[51] and sedentary time[52] as covariates, since physical activity is an important inductor of an anti-inflammatory environment and sedentary time is a risk factor for cardiovascular health, independent of physical activity levels. In addition, we only used the accurate food-frequency questionnaires, according to Goldberg's method.[25] Indeed, this method is useful in evaluating the mean population bias in reporting energy intake and recommends the use of information on physical activity, as in our study. Moreover, we include in our study important biological and lifestyle variables such as age, body mass index, sex, and smoking, all considered important modifiers of inflammatory biomarkers concentration.[1; 2] Another strength of our study is the use of a set of inflammatory biomarkers, rather than just one, as well as the calculation of an inflammatory biomarker score, a more complex and integrated assessment of low-grade inflammation. The inflammatory biomarkers, in general and in healthy people, are nonspecific pro-inflammatory response markers,[1] so this score considers the effect of all inflammatory biomarkers analysed.

This study has some limitations that should be acknowledged. We used a food-frequency questionnaire as an instrument to measure nutrient intake, and it may overestimate the food intake;[53] however, to overcome this limitation, we excluded 150 participants who were considered overreporters based on the Goldberg method.[25] Conversely, the food-frequency questionnaire is a good instrument to rank subjects by their level of consumption,[54] as we did by dividing the sample into three categories of fatty acid consumption. In addition, due of the lack of established cut-off points for inflammatory biomarkers for adolescents,[16] we used sex- and age-adjusted median values for create two categories of inflammatory state: lowest and highest, ranking the

sample according to inflammatory biomarkers. Indeed, the study of the relationship between dietary intakes inflammatory biomarkers in healthy populations is considered a difficult task.[16] Nevertheless, our cut-off points for IL-6 (1.9ng/L for most age/sex groups, but varying from 1.9 to 6.95ng/L) are very similar to the ones reported for the Asklepios Study (1.6 ng/L),[55] and the authors found an association with the dietary inflammation index. Our cut-off points for CRP (varying from 0.11 to 0.79mg/dL) are close to the ones reported by Visser et al. (0.22 mg/dL),[10] which showed a positive association between low-grade inflammation and being overweight, in children and adolescents.

In summary, high intakes of SFA, ALA, and EPA+DHA are the main dietary fatty acids related with a low-grade inflammation state in adolescents; on the one hand, SFA was positively associated with IL-6 and the overall inflammatory biomarkers score, and on the other hand, ALA and EPA+DHA were inversely associated with the overall inflammatory biomarkers score, independent of biological and lifestyle variables: body mass index, age, gender, pubertal stage, physical activity, sedentary time, socioeconomic status, and smoking habits. The other dietary fatty acids studied (MUFA, AA, LA, and TFA) were not predictive of low-grade inflammation.

Taken together, these findings support a high intake of vegetable and marine n-3 PUFA, that is, ALA and EPA+DHA, and a low consumption of SFA to prevent a low-grade inflammation state in adolescents. Longitudinal studies on low-grade inflammation and dietary fatty acid intake in adolescents are necessary to confirm or rule out our findings.

Acknowledgments: The authors gratefully acknowledged the participation of all adolescents and their parents, teachers and schools of the LabMed and Physical Activity Study. They also acknowledge the cooperation of volunteer's subjects, the Department of Hygiene and Epidemiology from Medical School (University of Porto) for the conversion the food frequency questionnaire data into nutrients, and the Research Centre in Physical Activity, Health and Leisure (University of Porto) for the sponsoring the LabMed and Physical Activity Study.

Financial Support: The research Centre in Physical Activity, Health and Leisure (University of Porto) is supported by the Portuguese Foundation for Science and

Technology (UID/DTP/00617/2013). J.AS. had been supported by program for advanced training (SFRH/PROTEC/68092/2010). R.S. has a Discovery Early Career Research Award from the Australian Research Council (DE150101921).

Conflicts of Interest: None.

Authorship: J.M., R.S., C.M., S.A., L.L. and P.M. designed the LabMed Physical Activity Study; J.AS., R.S., S.A., C.M. and L.L. collected the data; J.AS. and P.M. defined the research questions of the paper; J.AS. and P. M. analyzed the data; J.AS. wrote first version of the article; all authors reviewed and approved the final version of the manuscript. This paper is part of Almeida-de-Souza J PhD thesis in Faculty of Nutrition and Food Science, University of Porto, Porto, Portugal.

References

1. Minihane AM, Vinoy S, Russell WR *et al.* (2015) Low-grade inflammation, diet composition and health: current research evidence and its translation. *The British journal of nutrition* **114**, 999-1012.

2. Calder PC, Ahluwalia N, Albers R *et al.* (2013) A consideration of biomarkers to be used for evaluation of inflammation in human nutritional studies. *The British journal of nutrition* **109 Suppl 1**, S1-34.

3. Calder PC, Ahluwalia N, Brouns F *et al.* (2011) Dietary factors and low-grade inflammation in relation to overweight and obesity. *The British journal of nutrition* **106 Suppl 3**, S5-78.

4. van Greevenbroek MM, Schalkwijk CG, Stehouwer CD (2013) Obesity-associated low-grade inflammation in type 2 diabetes mellitus: causes and consequences. *The Netherlands journal of medicine* **71**, 174-187.

5. Spranger J, Kroke A, Mohlig M *et al.* (2003) Inflammatory cytokines and the risk to develop type 2 diabetes: results of the prospective population-based European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam Study. *Diabetes* **52**, 812-817.

6. Berg AH, Scherer PE (2005) Adipose tissue, inflammation, and cardiovascular disease. *Circulation research* **96**, 939-949.

7. Libby P (2012) History of Discovery: Inflammation in Atherosclerosis. *Arteriosclerosis, thrombosis, and vascular biology* **32**, 2045-2051.

8. Fernandes JV, Cobucci RN, Jatoba CA *et al.* (2015) The role of the mediators of inflammation in cancer development. *Pathology oncology research : POR* **21**, 527-534.

9. Ostan R, Lanzarini C, Pini E *et al.* (2015) Inflammaging and cancer: a challenge for the Mediterranean diet. *Nutrients* **7**, 2589-2621.

10. Visser M, Bouter LM, McQuillan GM *et al.* (2001) Low-grade systemic inflammation in overweight children. *Pediatrics* **107**, E13.

11. Ford ES, Galuska DA, Gillespie C *et al.* (2001) C-reactive protein and body mass index in children: findings from the Third National Health and Nutrition Examination Survey, 1988-1994. *The Journal of pediatrics* **138**, 486-492.

12. Lambert M, Delvin EE, Paradis G *et al.* (2004) C-reactive protein and features of the metabolic syndrome in a population-based sample of children and adolescents. *Clinical chemistry* **50**, 1762-1768.
13. Ford ES, Ajani UA, Mokdad AH (2005) The metabolic syndrome and concentrations of C-reactive protein among U.S. youth. *Diabetes care* **28**, 878-881.
14. Jarvisalo MJ, Harmoinen A, Hakanen M *et al.* (2002) Elevated serum C-reactive protein levels and early arterial changes in healthy children. *Arteriosclerosis, thrombosis, and vascular biology* **22**, 1323-1328.
15. Lee H, Lee IS, Choue R (2013) Obesity, inflammation and diet. *Pediatric gastroenterology, hepatology & nutrition* **16**, 143-152.
16. Warnberg J, Nova E, Romeo J *et al.* (2007) Lifestyle-related determinants of inflammation in adolescence. *The British journal of nutrition* **98 Suppl 1**, S116-120.
17. Carraro JC, Hermsdorff HH, Puchau B *et al.* (2015) Interleukin-6 is a better metabolic biomarker than interleukin-18 in young healthy adults. *Journal of physiology and biochemistry* **71**, 527-535.
18. Agostinis-Sobrinho C, Santos R, Moreira C *et al.* (2016) Association between serum adiponectin levels and muscular fitness in Portuguese adolescents: LabMed Physical Activity Study. *Nutrition, metabolism, and cardiovascular diseases : NMCD*.
19. Oliveira-Santos J, Santos R, Moreira C *et al.* (2016) Ability of Measures of Adiposity in Identifying Adverse Levels of Inflammatory and Metabolic Markers in Adolescents. *Childhood obesity (Print)* **12**, 135-143.
20. Lopes C, Aro A, Azevedo A *et al.* (2007) Intake and adipose tissue composition of fatty acids and risk of myocardial infarction in a male Portuguese community sample. *Journal of the American Dietetic Association* **107**, 276-286.
21. Silva D, Rego C, Guerra A (2004) Characterization of food habits and comparative study between two methods of food assessment in adolescents. *Rev Aliment Humana* **10**, 33–40.
22. Martins I, Porto A, Luísa O (2006) *Table Composition of Foods.* Lisbon, Portugal: National Institute of Health Dr Ricardo Jorge.
23. Ferreira F, Graça M (1985) *Composition Table of Portuguese Food*. 2nd ed. Lisbon, Portugal: National Institute of Health Dr Ricardo Jorge.
24. Goldberg GR, Black AE, Jebb SA *et al.* (1991) Critical evaluation of energy intake data using fundamental principles of energy physiology: 1. Derivation of cut-off limits to identify under-recording. *European journal of clinical nutrition* **45**, 569-581.
25. Black AE (2000) Critical evaluation of energy intake using the Goldberg cut-off for energy intake:basal metabolic rate. A practical guide to its calculation, use and limitations. *International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity* **24**, 1119-1130.
26. Schofield WN (1985) Predicting basal metabolic rate, new standards and review of previous work. *Human nutrition Clinical nutrition* **39 Suppl 1**, 5-41.
27. Trost SG, Ward DS, Moorehead SM *et al.* (1998) Validity of the computer science and applications (CSA) activity monitor in children. *Medicine and science in sports and exercise* **30**, 629-633.
28. Pietras SA, Goodman E (2013) Socioeconomic status gradients in inflammation in adolescence. *Psychosomatic medicine* **75**, 442-448.
29. Chiang JJ, Bower JE, Almeida DM *et al.* (2015) Socioeconomic status, daily affective and social experiences, and inflammation during adolescence. *Psychosomatic medicine* **77**, 256-266.

30. Brummett BH, Babyak MA, Singh A *et al.* (2013) Socioeconomic indices as independent correlates of C-reactive protein in the National Longitudinal Study of Adolescent Health. *Psychosomatic medicine* **75**, 882-893.
31. Rich C, Geraci M, Griffiths L *et al.* (2013) Quality Control Methods in Accelerometer Data Processing: Defining Minimum Wear Time. *PloS one* **8**, e67206.
32. Trost SG, Loprinzi PD, Moore R *et al.* (2011) Comparison of accelerometer cut points for predicting activity intensity in youth. *Medicine and science in sports and exercise* **43**, 1360-1368.
33. Lohmann TG, Roche AF, Martorell R (1988) *Anthropometric Standardization Reference Manual*: Human Kinetics Books, Champaign, Ill.
34. Cole TJ, Lobstein T (2012) Extended international (IOTF) body mass index cut-offs for thinness, overweight and obesity. *Pediatric obesity* **7**, 284-294.
35. Tanner JM, Whitehouse RH (1976) Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. *Archives of disease in childhood* **51**, 170-179.
36. Currie C, Molcho M, Boyce W *et al.* (2008) Researching health inequalities in adolescents: the development of the Health Behaviour in School-Aged Children (HBSC) family affluence scale. *Social science & medicine (1982)* **66**, 1429-1436.
37. World Health Organization (1998) *Guidelines for Controlling and Monitoring the Tobacco Epidemic.* Geneva: World Health Organization.
38. Kalogeropoulos N, Panagiotakos DB, Pitsavos C *et al.* (2010) Unsaturated fatty acids are inversely associated and n-6/n-3 ratios are positively related to inflammation and coagulation markers in plasma of apparently healthy adults. *Clinica chimica acta; international journal of clinical chemistry* **411**, 584-591.
39. Aeberli I, Molinari L, Spinas G *et al.* (2006) Dietary intakes of fat and antioxidant vitamins are predictors of subclinical inflammation in overweight Swiss children. *The American journal of clinical nutrition* **84**, 748-755.
40. Santos S, Oliveira A, Lopes C (2013) Systematic review of saturated fatty acids on inflammation and circulating levels of adipokines. *Nutrition research (New York, NY)* **33**, 687-695.
41. Poudel-Tandukar K, Nanri A, Matsushita Y *et al.* (2009) Dietary intakes of alpha-linolenic and linoleic acids are inversely associated with serum C-reactive protein levels among Japanese men. *Nutrition research (New York, NY)* **29**, 363-370.
42. Navarro SL, Kantor ED, Song X *et al.* (2016) Factors Associated with Multiple Biomarkers of Systemic Inflammation. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology* **25**, 521-531.
43. Kubota Y, Higashiyama A, Imano H *et al.* (2015) Serum Polyunsaturated Fatty Acid Composition and Serum High-Sensitivity C-Reactive Protein Levels in Healthy Japanese Residents: The KOBE Study. *The journal of nutrition, health & aging* **19**, 719-728.
44. Calder PC (2015) Marine omega-3 fatty acids and inflammatory processes: Effects, mechanisms and clinical relevance. *Biochimica et biophysica acta* **1851**, 469-484.
45. Burdge GC, Calder PC (2006) Dietary alpha-linolenic acid and health-related outcomes: a metabolic perspective. *Nutrition research reviews* **19**, 26-52.
46. Vannice G, Rasmussen H (2014) Position of the academy of nutrition and dietetics: dietary fatty acids for healthy adults. *Journal of the Academy of Nutrition and Dietetics* **114**, 136-153.
47. Prasad K (2009) Flaxseed and cardiovascular health. *Journal of cardiovascular pharmacology* **54**, 369-377.

515 48. Rajaram S (2014) Health benefits of plant-derived alpha-linolenic acid. *The American journal of clinical*
516 *nutrition* **100 Suppl 1**, 443s-448s.
517 49. Zhao N, Wang L, Guo N (2016) α-Linolenic acid increases the G 0/G 1 switch gene 2 mRNA
518 expression in peripheral blood mononuclear cells from obese patients: a pilot study. *Lipids in health and*
519 *disease* **15**, 1.
520 50. van Bussel BC, Henry RM, Ferreira I *et al.* (2015) A healthy diet is associated with less endothelial
521 dysfunction and less low-grade inflammation over a 7-year period in adults at risk of cardiovascular
522 disease. *The Journal of nutrition* **145**, 532-540.
523 51. Nimmo MA, Leggate M, Viana JL *et al.* (2013) The effect of physical activity on mediators of
524 inflammation. *Diabetes, obesity & metabolism* **15 Suppl 3**, 51-60.
525 52. Tremblay MS, Colley RC, Saunders TJ *et al.* (2010) Physiological and health implications of a
526 sedentary lifestyle. *Applied physiology, nutrition, and metabolism = Physiologie appliquee, nutrition et*
527 *metabolisme* **35**, 725-740.
528 53. Michels KB, Welch AA, Luben R *et al.* (2005) Measurement of Fruit and Vegetable Consumption with
529 Diet Questionnaires and Implications for Analyses and Interpretation. *American journal of epidemiology*
530 **161**, 987-994.
531 54. Agudo A (2005) Measuring intake of fruit and vegetables. In *FAO/WHO Workshop on Fruit and*
532 *Vegetable for Health, 1-3 September 2004, Kobe, Japan*: World Health Organization.
533 55. Shivappa N, Hebert JR, Rietzschel ER *et al.* (2015) Associations between dietary inflammatory index
534 and inflammatory markers in the Asklepios Study. *The British journal of nutrition* **113**, 665-671.
535

Table 1: Participants' characteristics according to sex among adolescents from the LabMed Activity Study

		All [a] (n=412)			Girls [a] (n=216)			Boys [a] (n=196)			P value [b]
		(%)			*(%)*			*(%)*			
Pubertal stage (Tanner A)	2	7.2			33.3			11.7			**0.001**
	3	33.0			54.6			36.2			
	4	47.1			12.0			38.8			
	5	12.6			2.8			13.3			
Pubertal stage (Tanner B)	2	7.0			22.7			11.7			**<0.001**
	3	20.6			46.8			18.4			
	4	50.2			27.8			54.1			
	5	22.1			3.2			15.8			
Body mass index	underweight	3.6			3.7			3.6			0.574
	normal weight	66.7			65.3			68.4			
	overweight	22.1			21.8			22.4			
	obese	7.5			9.3			5.6			
Smoking habits [d]	Current smokers	1.0			0.9			1.0			**0.037**
	Occasional smokers	1.7			1.4			2.0			
	Former smokers	7.8			4.6			11.2			
	Non-smokers	89.6			93.1			85.7			
		(Median)	*(25^{th})*	*(75^{th})*	*(Median)*	*(25^{th})*	*(75^{th})*	*(Median)*	*(25^{th})*	*(75^{th})*	
Age (years)		14.9	12.6	15.7	14.9	12.6	15.6	15.0	12.7	15.8	0.143
Socio-economic status		6.0	5.0	8.0	6.0	6.0	8.0	6.0	5.0	8.0	0.416
Sedentary Time [c] (minutes/day)		667.4	619.4	725.3	678.4	632.8	734.1	645.9	607.5	713.2	**0.003**
Moderate-to-vigorous physical activity [c] (minutes/day)		51.0	39.1	65.3	45.5	35.1	59.5	56.7	43.0	71.5	**<0.001**
Energy intake (kcal/day)		2 063	1 598	2 593	2 016	1 559	2 404	2 123	1 648	2 730	**0.005**
Protein (%en)		18.7	16.8	21.2	19.2	16.9	21.4	18.4	16.7	20.9	0.329
Total carbohydrates		50.2	45.4	54.2	49.9	45.0	54.3	50.4	46.3	54.2	0.546
Sugars (%en)		23.1	19.1	27.3	23.2	19.2	27.8	23.0	19.0	27.2	0.820
Dietary Fibre (g/1000kcal)		10.1	8.3	12.0	10.2	8.2	12.2	9.8	8.6	11.9	0.737
Total fat		32.5	29.5	35.3	32.8	29.2	35.4	32.3	29.9	35.2	0.644
SFA (%en)		10.4	9.3	11.4	10.3	9.2	11.5	10.4	9.5	11.3	0.580
MUFA (%en)		13.2	11.8	14.7	13.2	11.7	14.7	13.3 (–)	11.9	14.7	0.611

PUFA (%en)	5.78	5.03	6.50	5.78	4.92	6.68	5.77	5.09	6.37	0.954
n-6 (%en)	4.17	3.55	4.91	4.21	3.48	5.01	4.15	3.60	4.83	0.871
AA (%en)	0.06	0.05	0.09	0.06	0.05	0.09	0.06	0.05	0.09	0.853
LA (%en)	4.05	3.39	4.72	4.09	3.33	4.87	4.01	3.47	4.68	0.887
n-3 (%en)	0.57	0.49	0.68	0.57	0.50	0.68	0.57	0.47	0.67	0.402
ALA (%en)	0.46	0.41	0.52	0.46	0.41	0.52	0.46	0.41	0.51	0.529
EPA+DHA (%en)	0.16	0.10	0.26	0.16	0.11	0.26	0.15	0.10	0.25	0.367
TFA (%en)	0.44	0.33	0.54	0.42	0.32	0.54	0.45	0.35	0.53	0.064
Alcohol (%en) 45.4% of consumers [g]	0.00	0.00	0.33	0.00	0.00	0.28	0.13	0.00	0.47	**0.006**
CRP (mg/L)	0.20	0.11	0.77	0.11	0.11	0.49	0.34	0.11	1.26	**<0.001**
IL-6 (ng/L)	1.90	1.90	3.40	1.90	1.90	3.40	1.90	1.90	3.35	0.561
C3 (mg/dL)	116.0	107.0	126.5	119.0	107.0	127.0	115.0	106.5	126.0	0.888
C4 (mg/dL)	20.0	16.0	24.0	20.0	16.0	25.0	20.0	17.0	24.0	0.561
Overall inflammatory biomarkers score [f]	-0.55	-1.77	1.41	-0.51	-1.75	1.30	-0.59	-1.84	1. 43	0.844

Abbreviations: BMI – Body mass index, MVPA – moderate-to-vigorous physical activity, SFA – Saturated fatty acid, MUFA – Monounsaturated fatty acid, PUFA – Polyunsaturated fatty acid, n-6 – Omega 6, n-3 – Omega 3, AA – Araquidonic acid, LA – linoneic acid, ALA - Alpha-linolenic acid, EPA – Eicosapentaenoic acid, DHA – Docosahexaenoic acid, TFA - Trans fatty acid, CRP – C reactive protein, IL-6 – Interleukin 6, C3 – Complement component 3, C4 – Complement component 4, %en – energy intake percentage.

[a] The data shown in percentage for categorical variables and median (25^{th} -75^{th} percentiles) for continuous variables.

[b] P value was calculated based on Qui-square test for categorical variables, Student T test for continuous normally distributed variables and Mann-Whitney U test for skewed variables.

[c] Tanner A indicates development stages of breast in girls and genitalia in boys; Tanner B indicates development stages of public hair distribution in both sexes.

[d] It is the Family affluence scale, scored from 0 to 9 and higher scores indicated higher socioeconomic status.

[e] Because there is some missing values in these variables, n=329 (55.9% girls)

[f] Qui-squared test performed with “Current smokers” and “Occasional smokers” together to improve power of test.

[g] The alcohol consumers are adolescents that drink an alcoholic beverage at least once a month.

[h] The overall inflammatory biomarker score was designed summing the inflammatory biomarkers (CRP, IL-6, C3 and C4) categories, wherein for each category was assigned one point if the biomarker was above the median adjusted by age and sex or zero if below the median.

Table 2: Differences between nutrient intake tertiles and inflammatory biomarkers categories among adolescents from the LabMed Physical Activity Study.

		CRP (mg/L) [a]		IL-6 (ng/L) [a]		C 3 (mg/dL) [a]		C 4 (mg/dL) [a]		Overall inflammatory biomarker score [a, b]	
		lower	higher	lower	higher	lower	higher	lower	higher	lower	higher
SFA (%en)	≤9.72	33.3	30.0	35.7	24.8	31.9	31.7	33.6	29.7	34.1	30.0
	9.73 - 11.11	36.0	40.0	35.7	41.6	38.5	37.2	36.4	39.5	36.2	39.2
	≥11.12	30.6	30.0	28.5	33.6	29.6	31.2	30.0	30.8	29.7	30.8
MUFA (%en)	≤12.20	31.5	30.5	33.1	27.5	29.1	33.2	30.9	31.3	30.3	31.7
	12.21 - 14.15	36.5	32.6	35.0	34.2	39.4	29.6	37.8	31.3	37.8	32.2
	≥14.16	32.0	36.8	31.9	38.3	31.5	37.2	31.3	37.4	31.9	36.1
AA (%en)	≤0.05	30.6	34.2	32.7	31.5	33.3	31.2	29.5	35.4	31.4	33.0
	0.06 - 0.08	37.8	32.6	35.0	36.2	35.7	35.2	38.7	31.8	37.3	33.9
	≥0.09	31.5	33.2	32.3	32.2	31.0	33.7	31.8	32.8	31.4	33.0
LA (%en)	≤3.68	36.9	34.2	36.5	34.2	35.7	35.7	35.0	36.4	32.4	38.3
	3.69 - 4.64	33.8	37.4	36.5	33.6	35.7	35.2	37.8	32.8	37.8	33.5
	≥4.65	29.3	28.4	27.0	32.2	28.6	29.1	27.2	30.8	29.7	28.2
ALA (%en)	≤0.42	30.6	36.8	33.1	34.2	33.3	33.7	29.0	38.5	**27.0**	**38.8***
	0.43 - 0.49	34.2	28.9	33.1	29.5	34.3	29.1	35.5	27.7	**36.2**	**28.2**
	≥0.50	35.1	34.2	33.8	36.2	32.4	37.2	35.5	33.8	**36.8**	**33.0**
EPA+DHA (%en)	≤0.12	30.2	37.9	35.4	30.9	35.2	32.2	30.0	37.9	30.3	36.6
	0.13 - 0.21	32.0	31.6	28.5	37.6	30.0	33.7	32.7	30.8	29.7	33.5
	≥0.22	37.8	30.5	36.1	31.5	34.7	34.2	37.3	31.3	40.0	30.0
TFA (%en)	≤0.38	37.4	35.8	35.4	38.9	37.1	36.2	37.3	35.9	35.7	37.4
	0.39 - 0.50	30.2	32.1	31.9	29.5	31.5	30.7	30.0	32.3	30.8	31.3
	≥0.51	32.4	32.1	32.7	31.5	31.5	33.2	32.7	31.8	33.5	31.3

Abbreviations: CRP – C reactive protein, IL-6 – Interleukin 6, C3 – Complement component 3, C4 – Complement component 4, SFA – Saturated fatty acid, MUFA – Monounsaturated fatty acid, PUFA – Polyunsaturated fatty acid, n-3 – Omega 3, n-6 – Omega 6, LA – Linoleic acid, ALA - Alpha-Linolenic acid, EPA – Eicosapentaenoic acid, DHA – Docosahexaenoic acid, TFA – Trans fatty acid, %en – energy intake percentage.

[a] The data show column percentage by biomarkers categories and, when required, a symbol of qui-square statically differences between inflammatory biomarkers and nutrient intake: * <0.05.

[a] The overall inflammatory biomarker score was designed summing the inflammatory biomarkers (CRP, IL-6, C3 and C4) categories, wherein for each category was assigned one point if the biomarker was above the median adjusted by age and sex or zero if below the median.

Table 3: Fatty acids intake associated with higher inflammatory biomarkers among adolescents from the LabMed Physical Activity Study.

		CRP [a]		**IL-6** [a]		**C 3** [a]		**C 4** [a]		**Overall inflammatory biomarker score** [a, b]	
		OR (95% CI)	p_{trend}	OR (95% CI)	p_{trend}	OR (95% CI)	p_{trend}	OR (95% CI)	p_{trend}	OR (95% CI)	p_{trend}
SFA (%en)											
Crude models	≤9.72	1.00	0.798	**1.00**	**0.033**	1.00	0.925	1.00	0.507	1.00	0.282
	9.73 - 11.11	1.27 (0.76-2.12)		**1.87 (1.08-3.22)**		0.99 (0.59-1.64)		1.30 (0.78-2.17)		1.43 (0.85-2.40)	
	≥11.12	1.08 (0.60-1.95)		**1.98 (1.07-3.69)**		1.03 (0.57-1.84)		1.22 (0.68-2.21)		1.39 (0.77-2.51)	
Sex-adjusted models	≤9.72	1.00	0.790	**1.00**	**0.034**	1.00	0.928	1.00	0.509	1.00	0.284
	9.73 - 11.11	1.27 (0.76-2.12)		**1.88 (1.09-3.24)**		0.99 (0.60-1.64)		1.30 (0.78-2.18)		1.43 (0.85-2.40)	
	≥11.12	1.09 (0.60-1.96)		**1.98 (1.06-3.69)**		1.03 (0.57-1.84)		1.22 (0.68-2.21)		1.39 (0.77-2.51)	
Fully adjusted models [c]	≤9.72	1.00	0.183	1.00	0.051	1.00	0.271	1.00	0.207	**1.00**	**0.015**
	9.73 - 11.11	1.54 (0.83-2.86)		**2.42 (1.25-4.68)**		1.12 (0.59-2.11)		1.50 (0.82-2.75)		**2.18 (1.14-4.15)**	
	≥11.12	1.62 (0.79-3.32)		**2.16 (1.02-4.56)**		1.51 (0.73-3.15)		1.56 (0.78-3.11)		**2.51 (1.20-5.27)**	
MUFA (%en)											
Crude models	≤12.20	1.00	0.406	1.00	0.598	1.00	0.883	1.00	0.294	1.00	0.230
	12.21 - 14.15	0.93 (0.53-1.60)		1.07 (0.60-1.92)		0.63 (0.36-1.09)		0.88 (0.51-1.54)		0.97 (0.56-1.70)	
	≥14.16	1.25 (0.67-2.32)		1.19 (0.62-2.26)		0.96 (0.52-1.77)		1.32 (0.71-2.46)		1.41 (0.75-2.65)	
Sex-adjusted models	≤12.20	1.00	0.407	1.00	0.601	1.00	0.882	1.00	0.294	1.00	0.230
	12.21 - 14.15	0.92 (0.53-1.60)		1.07 (0.60-1.92)		0.63 (0.36-1.09)		0.88 (0.51-1.54)		0.97 (0.56-1.70)	
	≥14.16	1.25 (0.67-2.32)		1.18 (0.62-2.26)		0.96 (0.52-1.77)		1.32 (0.71-2.46)		1.41 (0.75-2.65)	
Fully adjusted models [c]	≤12.20	1.00	0.745	1.00	0.173	1.00	0.911	1.00	0.462	1.00	0.287
	12.21 - 14.15	0.90 (0.47-1.73)		1.43 (0.71-2.86)		0.79 (0.40-1.54)		0.90 (0.47-1.70)		1.10 (0.56-2.16)	
	≥14.16	1.10 (0.52-2.34)		1.75 (0.80-3.86)		0.93 (0.43-2.01)		1.26 (0.61-2.60)		1.51 (0.68-3.32)	

AA (%en)										
Crude models	≤0.05	1.00	0.517	1.00	0.871	1.00	0.521	1.00	0.741	1.00	0.186
	0.06 - 0.08	0.86 (0.52-1.44)		1.13 (0.67-1.92)		1.07 (0.65-1.77)		0.77 (0.46-1.27)		1.04 (0.62-1.75)	
	≥0.09	1.14 (0.66-1.98)		1.07 (0.60-1.91)		1.19 (0.69-2.07)		1.03 (0.59-1.79)		1.42 (0.81-2.50)	
Sex-adjusted models	≤0.05	1.00	0.523	1.00	0.853	1.00	0.519	1.00	0.740	1.00	0.185
	0.06 - 0.08	0.88 (0.53-1.46)		1.11 (0.65-1.89)		1.06 (0.64-1.77)		0.76 (0.46-1.27)		1.04 (0.62-1.74)	
	≥0.09	1.14 (0.66-1.99)		1.07 (0.60-1.91)		1.19 (0.69-2.07)		1.03 (0.59-1.79)		1.42 (0.81-2.50)	
Fully adjusted models [c]	≤0.05	1.00	0.671	1.00	0.761	1.00	0.921	1.00	0.804	1.00	0.249
	0.06 - 0.08	0.80 (0.43-1.47)		1.44 (0.77-2.68)		1.02 (0.54-1.92)		0.69 (0.38-1.24)		1.25 (0.66-2.35)	
	≥0.09	0.84 (0.42-1.65)		1.18 (0.59-2.37)		0.97 (0.48-1.97)		1.01 (0.52-1.93)		1.54 (0.75-3.16)	
LA (%en)										
Crude models	≤3.68	1.00	0.879	1.00	0.506	1.00	0.973	1.00	0.858	1.00	0.375
	3.69 - 4.64	1.21 (0.71-2.04)		0.96 (0.55-1.67)		1.08 (0.64-1.83)		0.85 (0.50-1.43)		0.74 (0.43-1.26)	
	≥4.65	0.99 (0.55-1.78)		1.20 (0.65-2.22)		1.02 (0.57-1.84)		1.03 (0.57-1.85)		0.74 (0.41-1.35)	
Sex-adjusted models	≤3.68	1.00	0.892	1.00	0.514	1.00	0.846	1.00	0.862	1.00	0.371
	3.69 - 4.64	1.20 (0.71-2.03)		0.97 (0.56-1.69)		1.09 (0.64-1.83)		0.85 (0.50-1.44)		0.74 (0.43-1.26)	
	≥4.65	0.99 (0.55-1.79)		1.20 (0.65-2.22)		1.02 (0.57-1.84)		1.02 (0.57-1.85)		0.74 (0.41-1.35)	
Fully adjusted models [c]	≤3.68	1.00	0.634	1.00	0.963	1.00	0.248	1.00	0.835	1.00	0.110
	3.69 - 4.64	1.09 (0.59-2.03)		0.86 (0.45-1.62)		1.14 (0.60-2.18)		0.92 (0.51-1.68)		0.64 (0.34-1.23)	
	≥4.65	0.85 (0.42-1.72)		1.00 (0.49-2.06)		0.67 (0.32-1.39)		0.93 (0.47-1.82)		0.54 (0.26-1.13)	
ALA (%en)										
Crude models	≤0.42	1.00	0.454	1.00	0.615	1.00	0.610	1.00	0.147	1.00	0.079
	0.43 - 0.49	0.69 (0.42-1.15)		0.76 (0.45-1.30)		0.83 (0.50-1.37)		0.61 (0.36-1.01)		**0.55 (0.33-0.92)**	
	≥0.50	0.80 (0.48-1.35)		0.86 (0.50-1.47)		1.12 (0.67-1.87)		0.67 (0.40-1.12)		0.60 (0.36-1.02)	

Sex-adjusted models	≤0.42	1.00	0.476	1.00	0.581	1.00	0.621	1.00	0.147	1.00	0.077
	0.43 - 0.49	0.69 (0.42-1.15)		0.76 (0.45-1.30)		0.83 (0.50-1.37)		0.61 (0.36-1.01)		**0.55 (0.33-0.92)**	
	≥0.50	0.81 (0.48-1.36)		0.85 (0.49-1.45)		1.12 (0.67-1.87)		0.67 (0.40-1.12)		0.60 (0.35-1.02)	
Fully adjusted models [c]	≤0.42	1.00	0.393	1.00	0.324	1.00	0.596	1.00	0.211	**1.00**	**0.047**
	0.43 - 0.49	0.70 (0.38-1.31)		0.56 (0.29-1.07)		0.60 (0.31-1.15)		0.61 (0.33-1.10)		**0.40 (0.21-0.77)**	
	≥0.50	0.75 (0.40-1.41)		0.70 (0.36-1.35)		1.14 (0.60-2.19)		0.67 (0.36-1.23)		**0.49 (0.26-0.95)**	
EPA+ DHA (%en)	Crude models										
Crude models	≤0.12	1.00	0.079	1.00	0.457	1.00	0.891	1.00	0.193	1.00	**0.035**
	0.13 - 0.21	0.82 (0.50-1.36)		1.51 (0.89-2.55)		1.20 (0.73-1.98)		0.81 (0.49-1.34)		0.96 (0.57-1.60)	
	≥0.22	0.62 (0.36-1.06)		0.90 (0.51-1.60)		1.01 (0.59-1.72)		0.69 (0.40-1.19)		0.58 (0.33-1.00)	
Sex-adjusted models	≤0.12	1.00	0.080	1.00	0.458	1.00	0.892	1.00	0.194	1.00	**0.035**
	0.13 - 0.21	0.83 (0.50-1.38)		1.50 (0.88-2.53)		1.20 (0.72-1.98)		0.81 (0.49-1.34)		0.96 (0.57-1.60)	
	≥0.22	0.62 (0.36-1.06)		0.90 (0.51-1.59)		1.01 (0.59-1.72)		0.69 (0.40-1.19)		0.58 (0.33-1.00)	
Fully adjusted models [c]	≤0.12	1.00	0.220	1.00	0.282	1.00	0.926	1.00	0.272	1.00	**0.025**
	0.13 - 0.21	0.63 (0.34-1.17)		1.50 (0.80-2.78)		1.19 (0.63-2.26)		0.72 (0.40-1.31)		0.82 (0.43-1.55)	
	≥0.22	0.63 (0.32-1.21)		0.76 (0.38-1.51)		1.07 (0.54-2.13)		0.68 (0.36-1.28)		**0.46 (0.23-0.93)**	
TFA (%en)											
Crude models	≤0.38	1.00	0.858	1.00	0.130	1.00	0.731	1.00	0.826	1.00	0.562
	0.39 - 0.50	1.09 (0.67-1.80)		0.75 (0.44-1.26)		1.02 (0.62-1.68)		1.14 (0.69-1.88)		0.98 (0.59-1.63)	
	≥0.51	1.02 (0.60-1.73)		0.67 (0.39-1.16)		1.11 (0.66-1.88)		1.02 (0.60-1.73)		0.83 (0.49-1.41)	
Sex-adjusted models	≤0.38	1.00	0.909	1.00	0.150	1.00	0.716	1.00	0.817	1.00	0.578
	0.39 - 0.50	1.07 (0.65-1.77)		0.77 (0.45-1.30)		1.03 (0.63-1.70)		1.15 (0.69-1.90)		0.99 (0.60-1.65)	
	≥0.51	1.01 (0.60-1.72)		0.68 (0.39-1.17)		1.11 (0.66-1.89)		1.02 (0.60-1.74)		0.83 (0.49-1.42)	
Fully adjusted models [c]	≤0.38	1.00	0.460	1.00	0.695	1.00	0.501	1.00	0.979	1.00	0.494
	0.39 - 0.50	1.21 (0.66-2.23)		0.92 (0.49-1.73)		1.17 (0.62-2.20)		1.09 (0.60-1.97)		0.87 (0.47-1.64)	
	≥0.51	1.25 (0.66-2.38)		0.88 (0.45-1.71)		1.25 (0.64-2.44)		0.96 (0.51-1.79)		0.79 (0.41-1.54)	

Abbreviations: OR – odds ratio, CI – confidence interval, CRP – C reactive protein, IL-6 – Interleukin 6, C3 – Complement component 3, C4 – Complement component 4, SFA – Saturated fatty acid, MUFA – Monounsaturated fatty acid, AA – Araquidonic acid, LA – linoneic acid, ALA – α-Linolenic acid, EPA – Eicosapentaenoic acid, DHA – Docosahexaenoic acid, TFA – Trans fatty acid, %en – percentage of energy intake.

[a] The data shows OR , 95 CI and p value for trend for each inflammatory biomarkers and overall inflammatory biomarker score as dependent variables. The predictor, that enter together in the model, were: SFA, MUFA, AA, LA, ALA, EPA+DHA, and TFA, all in percentage of energy intake.

[b] The overall inflammatory biomarker score was designed summing the inflammatory biomarkers (CRP, IL-6, C3 and C4) categories, wherein for each category was assigned one point if the biomarker was above the median adjusted by age and sex or zero if below the median.

[c] The fully adjusted models include also the following variables: gender, age, body mass index, pubertal stages – Tanner A and B, sedentary time, moderate-to-vigorous physical activity, smoking habits, socio-economic status. Because there is some missing values in sedentary time and moderate-to-vigorous physical activity variables, the fully adjusted models were performed with n=329 (55.9% girls).

7.2. PAPER II

'Five-a-day' fruit and vegetable intake is negatively associated with low-grade inflammation in adolescents: the LabMed Physical Activity Study.

[Submitted for publication]

‘Five-a-day’ fruit and vegetable intake is negatively associated with low-grade inflammation in adolescents: the LabMed Physical Activity Study

Authors (1st given names and, 2nd family names):

Juliana Almeida-de-Souza,[a,I,*] Rute Santos,[b,II] Luis Lopes,[c] Sandra Abreu,[d] Carla Moreira,[e,III] Rafaela Rosário,[f] Patrícia Padrão,[g,IV] Jorge Mota,[h] Pedro Moreira.[i,IV]

Affiliations:

[a] School of Health, Polytechnic Institute of Bragança, Av. D. Afonso V, 5300-121 Bragança, Portugal. julianaalmeida@ipb.pt.

[b] Faculty of Social Sciences, Early Start Research Institute, Building 21 Room 214, University of Wollongong, Northfields Avenue NSW 2522, Australia. rutes@uow.edu.au.

[c] Research Centre in Physical Activity, Health and Leisure, Faculty of Sport, University of Porto, , Rua Dr. Plácido Costa 91, 4200-450, Porto, Portugal. luis

[d] Research Centre in Physical Activity, Health and Leisure, Faculty of Sport, University of Porto, , Rua Dr. Plácido Costa 91, 4200-450, Porto, Portugal. sandra

[e] Research Centre in Physical Activity, Health and Leisure, Faculty of Sport, University of Porto, Rua Dr. Plácido Costa 91, 4200-450, Porto, Portugal. carla

[f] School of Nursing, University of Minho, Edifício dos Congregados, 4704-553 Braga, Portugal, rosaria

[g] Faculty of Nutrition and Food Sciences, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal, patriciapadrao@fcna.up.pt.

[h] Research Centre in Physical Activity, Health and Leisure, Faculty of Sport, University of Porto, Rua Dr. Plácido Costa 91, 4200-450, Porto, Portugal. jmota@fade.up.pt

[i] Faculty of Nutrition and Food Sciences, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal, pedromoreira@fcna.up.pt.

[I] Faculty of Nutrition and Food Sciences, University of Porto, Portugal, was also a filiation during this study.

[II] Research Centre in Physical Activity, Health and Leisure, Faculty of Sport, University of Porto, Portugal is a secondary filiation. University Institute of Maia, Portugal was also a filiation during this study.

[III] Health Sciences Research Unit-Nursing, Coimbra, Portugal.

[IV] Institute of Public Health, University of Porto, Porto, Portugal, is a secondary filiation.

***Corresponding author**: Juliana Almeida-de-Souza. School of Health, Polytechnic Institute of Bragança. Av. D. Afonso V – 5300-121 Bragança, Portugal. Telephone number: 273 330 950; Fax number: 273 327 915; e-mail: julianaalmeida@ipb.pt.

Disclosure statements: The authors disclose that LabMed Physical Activity Study was supported by Portuguese national funding agency for science, research and

technology (FCT) grants: BPD/102381/2014. The Research Centre on Physical Activity Health and Leisure (CIAFEL) is supported by UID/DTP/00617/2013 (FCT). This paper is part of Almeida-de-Souza J PhD thesis in Faculty of Nutrition and Food Science, University of Porto, Porto, Portugal. Almeida-de-Souza J had been supported by program for advanced training (SFRH/PROTEC/68092/2010). Santos R has a Discovery Early Career Award from the Australian Research Council (DE 150101921)

Conflict of interest statement: The authors declare there are no conflicts of interest.

Abstract

Background & aim: Five or more daily servings of fruit and vegetable intake are recommended, and the '5-a-day' is a public health message to encourage individuals to achieve this goal. Fruit and vegetable intake appears to be inversely associated with low-grade inflammation. However, most studies were conducted based on adult samples and used a single biomarker to measure low-grade inflammation. We aimed to study the association between fruit and vegetable intake and adherence to the 5-a-day recommendation, with low-grade inflammation in adolescents, as measured by a set of inflammatory biomarkers and an overall inflammatory biomarker score.

Methods: We conducted a cross-sectional study with 412 adolescents (52.4% girls), mean ages 14.4±1.7years, assessing categories of daily portion intake of fruits (<3 or ≥3 portions/day), vegetables (<3 or ≥3 portions/day), and vegetable soup (<2 or ≥2 portions/day), using a food-frequency questionnaire. To assess adherence to the 5-a-day recommendation, the previous continuum number of portions are totalled and categorized as <1, 1–5, or ≥5 portions/day. We collected blood samples to determine inflammatory biomarkers such as C-reactive protein (CRP), interleukin-6 (IL-6), complement component 3 (C3) and 4 (C4), and created categories of lower or higher for each biomarker, based on median values by sex and age. An overall inflammatory biomarker score was computed based on biomarker categories and classified as 0–1 or 2–4 biomarkers above the median. The odds ratio (OR) and 95% interval confidence (95%CI) were calculated from binary logistic regression, adjusted for confounders, to estimate the magnitude of association between food categories and inflammatory biomarkers.

Results: Adolescents with consumption of <2 portions/day of vegetable soup had a higher likelihood of having higher IL-6 (OR=12.95, 95%CI=1.63-102.84, $p<0.05$) when compared with consumption of ≥2 portions/day. An intake of ≥5 portions/day of fruits and vegetables compared to <1 portion/day was inversely associated with CRP (OR=5.24, 95%CI=1.62-16.97, $p_{trend}=0.005$), IL-6 (OR=5.36 95%CI=1.64-17.53, $p_{trend}=0.008$), C4 (OR=3.39, 95%CI=1.12-10.28, $p_{trend}=0.016$), and overall inflammatory biomarker score (OR=8.14, 95%CI=2.44-27.18, $p_{trend}<0.001$).

Conclusion: Inflammatory biomarkers were inversely associated with vegetable soup and ≥5 portions/day of fruits and vegetables. These findings support the current 5-a-day recommendation, in order to prevent low-grade inflammation in adolescence.

Key-words: C-reactive protein; Interleukin-6; Complement component 3; Complement component 4; Inflammatory biomarkers; vegetable soup; 5-a-day.

Introduction

Dietary guidelines recommend the daily consumption of five or more servings of fruits and vegetables to improve health and reduce the risk of chronic diseases [1, 2]. Similarly, the World Health Organization recommends a minimal consumption of 400g per day of fruits and vegetables [2] and suggests increasing individual intake up to 600g per day to reduce the global burden of cancer and cardiovascular diseases [3]. The "5-a-day" public health message is widespread and used in several countries to encourage individuals to achieve the goal of eating five or more daily servings of fruit and vegetable [2]. Adolescents' consumption of fruits and vegetables is low in much of Europe, including Portugal [4].

Many chronic diseases have been associated with low-grade inflammation [5, 6], including obesity, type 2 diabetes [7], cardiovascular diseases [8], and some cancers [9]. Moreover, in children and adolescents, low-grade inflammation has been associated with obesity [10], central obesity [11], metabolic syndrome [12], impaired endothelial function, and atherosclerosis [13].

Epidemiological evidence from studies based on adult samples has shown that a high intake of fruits and vegetables appears to be inversely associated with low-grade inflammation [5]. However, data regarding children [14] and adolescents [15] is scarce. Inflammatory status is heavily reliant on the measurement of the inflammatory biomarkers, such as acute phase proteins and cytokines [6]. In addition, most studies assessed a single biomarker as a measure of low-grade inflammation to study its association with fruit and vegetable intake, especially C-reactive protein (CRP) [5]. Some studies have included interleukin-6 (IL-6) as well, but the role of complement component 3 (C3) and 4 (C4) remains unknown [5]. Until now, the set of biomarkers

that fully describes low-grade inflammation has not been identified [6], and therefore, approaches that include a set of inflammatory biomarkers [15, 16] or an inflammatory biomarker score [17] seem sensible.

In this context, the present study aimed to analyse the association between intakes of fruits, vegetables, vegetable soup, and 5-a-day with a set of inflammatory biomarkers and an overall inflammatory biomarker score in adolescents.

Materials and Methods

Study design and sampling

This study analysis baseline data from the Longitudinal Analysis of Biomarkers and Environmental Determinants of Physical Activity Study (LabMed Physical Activity Study), collected in the Fall of 2011. Briefly, study recruitment was conducted at all participating schools that have collaboration agreements previously established with our research centre. A sample size was estimated in 1086 subjects, according to the exposure of combined healthy diet/physical activity pattern prevalence (14% [18]), to detect 15% difference between exposed and unexposed, for have a power of 0.80, a 2-tailed significance of 5%, and an expected dropout rate of 20%. All students from 7^{th} and 10^{th} grade were invited (N=1678). Both adolescents and their parents/guardians that returned written informed consent (n=1229) entry in the study, and all data was collected in schools.

From an initial sample of apparently healthy adolescents (12–18 years old), 534 accepted to undergo blood sampling and 622 had accurate data on dietary intake (excluding the misreporting as explained in the dietary intake section), therefore the final sample of the present study comprises 412 adolescents (216 girls), aged 14.4±1.7

years old. We found no differences in most variables between those who were selected or excluded from statistical analysis. However, in the group of subjects that were more excluded, there were higher frequencies of boys, smokers, subjects with underweight, and with lower energy intake. The final models were adjusted for these variables.

Ethical statement

We conducted the study in accordance with the Helsinki declaration for Human Studies. The Portuguese Data Protection Authority (#1112434/2011), the Portuguese Ministry of Science and Education (0246200001/2011), and Faculty of Sport, University of Porto approved the study.

Inflammatory biomarkers and overall inflammatory biomarker score

Blood samples were collected only in adolescents with no known disease and after at least ten hours of fasting. Samples were refrigerated (4º-8°C), and sent for analysis of inflammatory biomarkers at the laboratory. High sensitive CRP by the Latex-enhanced turbidimetric assay (Siemens Advia 1600/1800, Erlangen, Germany); IL-6 was determined by the Chemiluminescence immunoassay (Immulite 2000, Diagnostic Products Corporation, Los Angeles, CA); and C3 and 4 C4 were determined by the Immunoturbidimetric assay (Siemens Advia 1600/1800, Erlangen, German).

Participants were grouped in two categories, higher or lower (inflammatory state), based on medians values adjusted by age and sex, considering that increasing of biomarkers in low-grade inflammation can be minimal [6] and no biologic cut-points are established for adolescents. Medians of each inflammatory biomarker category

(higher/lower) were 0.11/0.92 mg/L for CRP, 1.90/4.20 ng/L for IL-6, 107.00/127.00 mg/dL for C3, and 17.00/25.55 mg/dL for C4.

We created an overall inflammatory biomarker score assigning one point to those who were above the sex-age-adjusted medians or zero for those below, for each inflammatory biomarker, and then summing all points assigned. The overall inflammatory biomarker score varies from zero to four points. Two categories were defined: 0-1 (49.9%) or 2-4 (50.1%) inflammatory biomarkers above the median.

Physical activity and sedentary time

We used accelerometers GT1M Actigraph (ActiGraph, Pensacola, Florida, USA), a lightweight and biaxial monitor, to determine physical activity and sedentary time. Participants were instructed to tightly attach it on the right side of hip, with the notch faced upwards, and to use it for five consecutive days (three weekdays and two weekend days), during waking hours and remove it during water-based activities. The epoch length was set to 2 seconds to allow a more detailed estimate of physical activity intensity.

We worked with an automated data reduction program (ActivLive 6.12, ActiGraph, Pensacola, Florida, USA) to analyse accelerometer data. Periods of 60 minutes of consecutive zeros were signalled as non-wear time. Participants had to have at least two weekdays and one weekend with a minimum of 8 hours/day of accelerometer wear time to be included in the analysis.

After screening, the time spent in different physical activity intensities were determined by the raw activity “counts”, considering Evensons et al cut-points.

133 Physical activity were expressed in mean counts/min and also in estimates of the time
134 spent (in minutes/day) in moderate-to-vigorous physical activity and sedentary time.
135
136 **Dietary intake**
137 We use a self-administered semi-quantitative food-frequency questionnaire validated
138 for the Portuguese population [19], and adapted to adolescents[20], to measure the
139 dietary intake. This food-frequency questionnaire assesses the food habits in the
140 previous 12 months and lists 91 food and beverage items or categories, with nine
141 frequency possibilities (from "never or less than once per month" to "six or more
142 times per day"), with a standard portion sizes, and with a seasonality option. In the
143 questionnaire there is still space available for each participant to include any food not
144 listed. Dietary intake quantification was performed, for each option selected,
145 multiplying the portion size in grams by the multiple/fraction of daily frequency
146 intake and by a seasonality variation factor. Food to energy and nutrients intake
147 conversion was performed using The Food Processor Plus program version SQL
148 (ESHA Research, Salem, OR, USA). This database was supplemented with the
149 Portuguese food composition databases.
150 We excluded food-frequency questionnaires with implausible energy intake by
151 determination of dietary assessment misreporting, using the Goldberg cut-off method,
152 adapted by Black [21]. Briefly, basal metabolic rate, by Schofield equation, and a
153 ratio energy intake/basal metabolic rate was calculated to compare with 95%
154 confidence limits (cut-offs). The cut-offs were calculated using: 1st) mean physical
155 activity level of 1.23 was calculated considering accelerometers data and Trost
156 equation; 2nd) 21 days of dietary assessment was considered, as recommended for
157 food-frequency questionnaire data [21]; 3rd) within-subject coefficient of variation in

158 energy intake was calculated considering mean and standard deviation in our sample
159 and numbers of days of dietary assessment; 4th) between-subject variation in physical
160 activity was calculated considering mean and standard deviation in our sample, to be
161 more precise, avoiding tabulated theoretical values; 5th) coefficient of variation in
162 basal metabolism rate of 8.5% was considered as recommendation [21]. We achieved
163 0.61–2.48 cut-offs; therefore, adolescents with energy intake/basal metabolic rate out
164 of this range were excluded (n=150) from the statistical analysis.
165 To quantify the number of portion from food categories (fruits, vegetables and
166 vegetable soup), we summed all items from each food group, and divided them by the
167 mean portion size (160g for fruit or vegetable, 80g for green pulses), according to
168 Food Guide for the Portuguese Population.[1] For fruit category, we included
169 apple/pear, orange/tangerine, banana, kiwi, strawberry, cherry, peach/plum,
170 melon/watermelon, persimmon, fig/medlar/apricot, grape and papaya/mango; and
171 excluded canned fruit in syrup and juice fruit, for the unknown added sugar content
172 on those foods. For vegetable category, we considered white/savoy cabbages,
173 bunch/portuguese cabbages, curly kale, broccoli, cauliflower/brussel sprout, turnip
174 sprout/turnip greens/spinach, green been, lettuce/watercress, onion, carrot, turnip
175 white, tomato, pepper, cucumber; as well green pulses in item peas/broad beans; and
176 excluded potatoes and dried pulses for its starch content very different from others
177 foods in this group and because there is not consensus about inclusion of those foods
178 in the vegetable category [22]. Vegetable soup portion size considered was 295g, and
179 it was treated as a different food category because it is a single item from food-
180 frequency questionnaire. Vegetable soup is considered an important source of
181 vegetable intake in Portuguese diet, being consumed mostly as a starter of the main

meals (lunch and dinner), and recommendation of two portions/day is usually recognized as an important Portuguese healthy dietary guideline.

Participants were grouped according to daily portion intake, into low or adequate, of fruits (<3 or ≥3 portions/day), vegetables (<3 or ≥3 portions/day), and vegetable soup (<2 or ≥2 portions/day), in accordance with Portuguese guidelines.[1] Also, we summed fruit, vegetable and vegetable soup portion numbers, to analyse adherence to the 5-a-day recommendation, and grouped the participants in very low (<1 portion/day), low (≥1 to <5 portions/day), or adequate (≥5 portions/day) fruit and vegetable consumptions.

Others assessments

Height and weight were measured with a portable stadiometer (SECA 213, Hamburg, Germany) and, with a portable electronic weight scale (TANITA Inner Scan BC532, Tokyo, Japan), respectively. During the measurements, participants were standing upright, lightly dressed and without shoes. The body mass index was calculated from the ratio of weight (kg) to height squared (m^2) and participants were classified according to Cole's body mass index cut-offs.

Pubertal stage ranging from 1 to 5 was self-assessed relatively to the secondary sex characteristics, according to the criteria of Tanner and Whitehouse. Tanner A indicates the stage of breast development in girls and genitalia development (penis size and testicular volume) in boys; and Tanner B indicates the stage of public hair distribution, in both sexes.

The participants' socio-economic status was identified by the Family Affluence Scale. This is a self-filled questionnaire considering things about adolescents' family (number of car, bedrooms, vacations, computers…) and ranks the participants from 0

to 9 (lower to highest socio-economic status).

Smoking habits were self-reported and participants were classified according to the World Health Organization criteria as follows: non-smokers, former smokers, occasional smokers, and current smokers.

Statistical analyses

Participants' characteristics are presented as percentages for categorical variables; and as medians, 25^{th} and 75^{th} percentiles for continuous variables. Mann-Whitney U test and Qui-square test were used to assess sex differences in continuous and categorical variables, respectively.

Binary logistic regression models were constructed to predict inflammatory biomarkers and the overall inflammatory biomarker score. There were four models for each inflammatory biomarker and for the overall inflammatory biomarker score (as dependent variables) and food groups portion categories (as predictors), using the major portion as reference: Model 1 – crude model; Model 2 – adjusted for sex; Model 3 – adjusted for sex, age, pubertal stage (Tanner A and B), body mass index, energy intake, socio-economic status, sedentary time, moderate-to-vigorous physical activity and smoking habits; Model 4 – adjusted for the same variables as model 3 plus the other food group portions (for example: vegetable portion and fruit portions for models with vegetable soup portions predictor).

Binary logistic regression was also performed to calculate odds ratio (OR), 95% confidence interval (CI), and p for trend, predicting the odds of having a higher inflammatory biomarker and overall inflammatory biomarker score for the 5-a-day recommendation. Three models were constructed: Model 1 – crude model; Model 2 –

231 adjusted for sex; Model 3 – adjusted for sex, age, pubertal stage (Tanner A and B),
232 body mass index, energy intake, socio-economic status, sedentary time, moderate-to-
233 vigorous physical activity and smoking habits.

234 Multicollinearity tests were performed, and we detected no multicollinearity between
235 independent variables.

236 Post hoc power calculations were performed considering the smaller sample size
237 (n=329), the smaller odds ratio for fully adjusted model (OR=2.44), a null hypothesis
238 value of 0.5, a significance level of 0.05, and achieving a power of 0.97.

239 A 0.05 level of significance and 95%CI (confidence interval) were considered. Data
240 analysis was performed using the statistical package SPSS®, version 21.0 (SPSS Inc.,
241 Chicago, IL, USA) and sample power was calculated using G*power, version 3.1
242 (Faul, Erdfelder, Lang, & Buchner, 2007).
243

244 Results

245 The sample characteristics are presented in table 1. Girls presented on average a lower
246 CRP, less moderate-to-vigorous physical activity and higher sedentary time and
247 vegetable soup intake than boys ($p<0.05$ for all).
248

249 Adolescents with consumption of ≥2 portion/day of vegetable soup had a lower
250 prevalence of having higher IL-6. No significant differences were observed between
251 the other inflammatory biomarkers or overall inflammatory biomarker score and fruit,
252 vegetable or vegetable soup categories (table 2).
253

Adolescents with consumption of <2 portions/day of vegetable soup had a significantly higher likelihood of having higher IL-6 when compared with consumption of ≥2 portions/day. (table 3).

Adolescents with a low intake (<1 portion/day) of fruit and vegetable, when compared with 5-a-day recommendation adherence (≥5 portions/day), showed a significantly high likelihood of having higher CRP, IL-6, C4 and overall inflammatory biomarker score, p for trend <0.05 for all, in fully adjusted models (table 4).

Discussion

The main finding of this study suggests that the consumption of five or more portions daily of fruits and vegetables is inversely associated with CRP, IL-6, C4 and an overall inflammatory biomarker score after adjusting the analysis for several potential confounders. These results support the adherence to the 5-a-day recommendation for the prevention of low-grade inflammation state, considered an important contributor for development of a range of chronic conditions. To the best of our knowledge, no prior study has specifically analyzed the association between low-grade inflammation and fruit and vegetable intake based on adherence to the 5-a-day recommendation. Nevertheless, our results align with those of other studies conducted in adolescents and adults that also showed an inverse association between fruit and vegetable intake and low-grade inflammation [15, 16].

It is interesting to note that, in our study, the inverse association between low-grade inflammation and five or more portions of fruit and vegetable daily compared with fruits, vegetables or vegetable soup, per se, showed a stronger association with low-

278 grade inflammation. This result shows the importance of the 5-a-day recommendation
279 and its impact on most inflammatory biomarkers studied. Besides the quantity itself (3
280 serving of fruits or vegetables and 2 for vegetable soup versus 5 serving of these
281 combined), the synergistic effect between food compounds may explain the stronger
282 association with inflammation. A previous review found that antioxidant vitamins
283 such C, E, and carotenoids have been identified as having an anti-inflammatory effect
284 [23]. However, Kaulmann and Bohn [24] justified the absence of this synergistic
285 effect as one reason for the lack of association or a positive association with risk of
286 disease in several experimental studies using those antioxidants as supplementation
287 and not the whole food. Yet, Smidowicz and Regula [25] in a review study that
288 included IL-6 and CRP inflammatory biomarkers concluded that the Mediterranean
289 diet and plant-based diet models (both characterized by abundant fruits, vegetables,
290 and other whole foods) provide anti-inflammatory properties. Nevertheless, the
291 relationship between carotenoids and inflammation remains poorly understood [24].

292 Unlike the case of CRP, research on the association between fruits and vegetables and
293 other biomarkers is scarce. In our study, we included a set of other inflammatory
294 biomarkers. Our results show an inverse association between IL-6 and vegetable soup.
295 That is, the daily consumption of less than 2 portions of vegetable soup can increase
296 by about 13 times the likelihood of having higher IL-6 (fully adjusted model).
297 Although it is known that the odds ratio overstates the relative risk by less than 50%
298 for a wide range of both initial risks and effect sizes, any qualitative judgment is
299 unaltered by this discrepancy [26]. Therefore, our finding seems to be highly
300 significant considering the Portuguese culture and dietary habits. As previously
301 showed in a sample of Portuguese adults, vegetable soup, lettuce/watercress and
302 tomato represent almost 50% of the weekly mean consumption of vegetables [27].

303 Soup is recognized as having high-satiety properties [28], and soup consumption was
304 associated with lower energy [28, 29] and fat intake [29]. Considering that high-fat
305 meals may increase non-fasting IL-6 [30], the protective effects of vegetable soup on
306 that biomarker may also be related to the lower-fat intake in those who consume lager
307 amounts of vegetable soup. To the best of our knowledge, no other observational
308 study found the relationship between vegetable soup and IL-6. Sanchez-Moreno et al.
309 [31] conducted an experimental study in adults in which no significant changes were
310 observed for participants with an intake of 500ml of pressurized soup during 14 days
311 on IL-6, but a beneficial effect for CRP was reported. Other studies in adults [32, 33]
312 that examined the relationships between soup intake and CRP found no association,
313 which aligns with our results. Yet, and in agreement to our findings, Asgard et al. [34]
314 showed that a reduction in IL-6 seems to be more sensitive than in CRP to increase
315 plasma antioxidant properties. IL-6 was inversely associated with four plasma
316 carotenoids, while CRP was associated with only one. Also worthy of note is that IL-
317 6 is an important factor for acute phase-protein hepatic synthesis [6] such as CRP, C3
318 and C4; therefore, it can be argued that in a young and healthy population, IL-6 can
319 be the first manifestation of low-grade inflammation.

320 The strengths of this study include the novelty of its aim and the use of objectively
321 measured physical activity and sedentary time as covariates, once sedentary time has
322 been previously considered a risk factor for cardiovascular health independently of
323 physical activity levels [35]. In addition, we used only accurate food-frequency
324 questionnaires, accordingly to Goldberg's method [21]. Indeed, this method is useful
325 for evaluating mean population bias in the reporting of energy intake in several age
326 groups and recommends the use of information about physical activity, as we did in
327 the current study. Moreover, our models considered other important potential

confounders such as age, body mass index, sex, and smoking that have been shown to be important modifiers of inflammatory biomarker concentration [5, 6]. Other strengths are the evaluation of the C3 and C4 biomarkers, the roles of which are still unknown in the relationship between diet and inflammation, and of a set of inflammatory biomarkers rather than only one as well as the calculation of an inflammatory biomarker score, a more complex and integrated assessment of low-grade inflammation. Van Bussel et al. [17] also used a biomarker score, but in a different combination. The inflammatory biomarkers, in general and in healthy people, are non-specific pro-inflammatory response markers [6], so our score attempted to combine the effects of all inflammatory biomarkers analyzed.

This study has several limitations to be acknowledged. First, we used a food-frequency questionnaire instrument to measure fruit and vegetable intake, and it is known that this instrument may overestimate the food intake; however, to overcome this limitation we excluded 150 participants who were considered over-reporters based on the Goldberg's method [21]. Conversely, the food-frequency questionnaire is a useful instrument for ranking subjects by their level of food consumption, as we did by dividing the sample into three categories of consumption of fruits and vegetables and by using the 5-a-day recommendation cut-offs. Second, because of the lack of established biologic cut-off points for inflammatory biomarkers for adolescents, we used median values adjusted for age and sex in order to create two categories of inflammatory states. The study of the relationship between dietary intakes and inflammatory biomarkers in healthy populations is considered a difficult task [28]; nevertheless, our cut-off points for IL-6 (1.9ng/L for most age/sex groups, varying from 1.9 to 6.95ng/L) are very similar to the those reported in the Asklepios Study (1.6 ng/L) [36], and the authors found an association between the dietary

353 inflammation index and IL-6. Furthermore, our cut-off points for CRP (varying from
354 0.11 to 0.79mg/dL) is very close that of Visser et al. (0.22 mg/dL) [10], which found
355 an association between low-grade inflammation and overweight in children and
356 adolescents. In addition to food consumption, also confounding factors were based on
357 self-reports, namely, pubertal stage, which always includes a chance of bias.

358 In summary, vegetable soup intake was inversely associated with IL-6 after
359 adjustments for several biological and lifestyle confounders. Fruit or vegetable intake,
360 treated alone, was not associated with any inflammatory biomarker or overall
361 inflammatory biomarker score. Five or more portions of fruit and vegetable intake,
362 when compared with less than one, were inversely associated with CRP, IL-6, C4 and
363 overall inflammatory biomarker score after adjustments for biological and lifestyle
364 characteristics. Taken together these findings support the 5-a-day fruit and vegetable
365 intake recommendation to prevent a state of low-grade inflammation in adolescents.
366 Future studies using longitudinal methodology are important to confirm or rule out
367 our findings.

368

369 ## Acknowledgments

370 The authors gratefully acknowledged the participation of all adolescents and their
371 parents, teachers and schools of the LabMed and Physical Activity Study; the
372 cooperation of volunteer's; the Department of Hygiene and Epidemiology (University
373 of Porto) for the conversion from food-frequency questionnaire data into nutrients,
374 and the Research Centre in Physical Activity, Health and Leisure (University of
375 Porto) for the sponsoring the LabMed and Physical Activity Study.

376

Statement of authorship

The authors' contributions are as follows: J. M., R. S., C. M., S. A., L. L. and P. M. designed the LabMed Physical Activity Study; J. AS., R. S., S. A., C. M., L. L. and R. R. collected the data; J. AS. and P. M. defined the research questions of the paper; J. AS., P. P. and P. M. analysed the data; J. AS. wrote first version of the article; all authors reviewed and approved the final version of the manuscript. This paper is part of J. AS. PhD thesis in Faculty of Nutrition and Food Sciences, University of Porto, Porto, Portugal.

Conflict of interest statement

There are no conflicts of interest to declare.

Funding source

This work was supported by Portuguese national funding agency for science, research and technology (FCT) [grant: UID/DTP/00617/2013]; [grant: BPD/102381/2014]; J. AS., had been supported by a program for advanced training [grant: SFRH/PROTEC/68092/2010]. R. S. has a Discovery Early Career Award from the Australian Research Council (ARC) [grant: DE 150101921].

References

[1] Rodrigues SS, Franchini B, Graca P, de Almeida MD. A new food guide for the Portuguese population: development and technical considerations. Journal of nutrition education and behavior. 2006;38:189-95.

[2] World Health Organization. Fruit and vegetables for health: report of a Joint FAO/WHO Workshop, 1-3 September, 2004, Kobe, Japan. Fruit and vegetables for health: Report of a Joint FAO/WHO Workshop, 1-3 September, 2004, Kobe, Japan: World Health Organization; 2005.

[3] Lock K, Pomerleau J, Causer L, Altmann DR, McKee M. The global burden of disease attributable to low consumption of fruit and vegetables: implications for the global strategy on diet. Bulletin of the World Health Organization. 2005;83:100-8.

[4] Lynch C, Kristjansdottir AG, Te Velde SJ, Lien N, Roos E, Thorsdottir I, et al. Fruit and vegetable consumption in a sample of 11-year-old children in ten European countries--the PRO GREENS cross-sectional survey. Public health nutrition. 2014;17:2436-44.

[5] Calder PC, Ahluwalia N, Brouns F, Buetler T, Clement K, Cunningham K, et al. Dietary factors and low-grade inflammation in relation to overweight and obesity. The British journal of nutrition. 2011;106 Suppl 3:S5-78.

[6] Calder PC, Ahluwalia N, Albers R, Bosco N, Bourdet-Sicard R, Haller D, et al. A consideration of biomarkers to be used for evaluation of inflammation in human nutritional studies. The British journal of nutrition. 2013;109 Suppl 1:S1-34.

[7] van Greevenbroek MM, Schalkwijk CG, Stehouwer CD. Obesity-associated low-grade inflammation in type 2 diabetes mellitus: causes and consequences. The Netherlands journal of medicine. 2013;71:174-87.

[8] Libby P. History of Discovery: Inflammation in Atherosclerosis. Arteriosclerosis, thrombosis, and vascular biology. 2012;32:2045-51.

[9] Fernandes JV, Cobucci RN, Jatoba CA, Fernandes TA, de Azevedo JW, de Araujo JM. The role of the mediators of inflammation in cancer development. Pathology oncology research : POR. 2015;21:527-34.

[10] Visser M, Bouter LM, McQuillan GM, Wener MH, Harris TB. Low-grade systemic inflammation in overweight children. Pediatrics. 2001;107:E13.

[11] Warnberg J, Nova E, Moreno LA, Romeo J, Mesana MI, Ruiz JR, et al. Inflammatory proteins are related to total and abdominal adiposity in a healthy adolescent population: the AVENA Study. The American journal of clinical nutrition. 2006;84:505-12.

[12] Ford ES, Ajani UA, Mokdad AH. The metabolic syndrome and concentrations of C-reactive protein among U.S. youth. Diabetes care. 2005;28:878-81.

[13] Jarvisalo MJ, Harmoinen A, Hakanen M, Paakkunainen U, Viikari J, Hartiala J, et al. Elevated serum C-reactive protein levels and early arterial changes in healthy children. Arteriosclerosis, thrombosis, and vascular biology. 2002;22:1323-8.

[14] Qureshi MM, Singer MR, Moore LL. A cross-sectional study of food group intake and C-reactive protein among children. Nutrition & metabolism. 2009;6:40.

[15] Holt EM, Steffen LM, Moran A, Basu S, Steinberger J, Ross JA, et al. Fruit and vegetable consumption and its relation to markers of inflammation and oxidative

stress in adolescents. Journal of the American Dietetic Association. 2009;109:414-21.

[16] Root MM, McGinn MC, Nieman DC, Henson DA, Heinz SA, Shanely RA, et al. Combined fruit and vegetable intake is correlated with improved inflammatory and oxidant status from a cross-sectional study in a community setting. Nutrients. 2012;4:29-41.

[17] van Bussel BC, Henry RM, Ferreira I, van Greevenbroek MM, van der Kallen CJ, Twisk JW, et al. A healthy diet is associated with less endothelial dysfunction and less low-grade inflammation over a 7-year period in adults at risk of cardiovascular disease. The Journal of nutrition. 2015;145:532-40.

[18] Huh J, Riggs NR, Spruijt-Metz D, Chou CP, Huang Z, Pentz M. Identifying patterns of eating and physical activity in children: a latent class analysis of obesity risk. Obesity (Silver Spring, Md). 2011;19:652-8.

[19] Lopes C, Aro A, Azevedo A, Ramos E, Barros H. Intake and adipose tissue composition of fatty acids and risk of myocardial infarction in a male Portuguese community sample. Journal of the American Dietetic Association. 2007;107:276-86.

[20] Silva D, Rego C, Guerra A. Characterization of food habits and comparative study between two methods of food assessment in adolescents. Rev Aliment Humana. 2004;10:33–40.

[21] Black AE. Critical evaluation of energy intake using the Goldberg cut-off for energy intake:basal metabolic rate. A practical guide to its calculation, use and limitations. International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity. 2000;24:1119-30.

[22] Roark RA, Niederhauser VP. Fruit and vegetable intake: issues with definition and measurement. Public health nutrition. 2013;16:2-7.

[23] Shivappa N, Steck SE, Hurley TG, Hussey JR, Hebert JR. Designing and developing a literature-derived, population-based dietary inflammatory index. Public health nutrition. 2014;17:1689-96.

[24] Kaulmann A, Bohn T. Carotenoids, inflammation, and oxidative stress--implications of cellular signaling pathways and relation to chronic disease prevention. Nutrition research (New York, NY). 2014;34:907-29.

[25] Smidowicz A, Regula J. Effect of nutritional status and dietary patterns on human serum C-reactive protein and interleukin-6 concentrations. Advances in nutrition (Bethesda, Md). 2015;6:738-47.

[26] Davies HTO, Crombie IK, Tavakoli M. When can odds ratios mislead? BMJ : British Medical Journal. 1998;316:989-91.

[27] Oliveira A, Maia B, Lopes C. Determinants of inadequate fruit and vegetable consumption amongst Portuguese adults. Journal of human nutrition and dietetics : the official journal of the British Dietetic Association. 2014;27 Suppl 2:194-203.

[28] Mattes R. Soup and satiety. Physiology & behavior. 2005;83:739-47.

[29] Zhu Y, Hollis JH. Soup consumption is associated with a lower dietary energy density and a better diet quality in US adults. The British journal of nutrition. 2014;111:1474-80.

[30] Nappo F, Esposito K, Cioffi M, Giugliano G, Molinari AM, Paolisso G, et al. Postprandial endothelial activation in healthy subjects and in type 2 diabetic patients: role of fat and carbohydrate meals. J Am Coll Cardiol. 2002;39:1145-50.

[31] Sanchez-Moreno C, Cano MP, de Ancos B, Plaza L, Olmedilla B, Granado F, et al. Consumption of high-pressurized vegetable soup increases plasma vitamin C and decreases oxidative stress and inflammatory biomarkers in healthy humans. The Journal of nutrition. 2004;134:3021-5.

[32] Esmaillzadeh A, Kimiagar M, Mehrabi Y, Azadbakht L, Hu FB, Willett WC. Fruit and vegetable intakes, C-reactive protein, and the metabolic syndrome. The American journal of clinical nutrition. 2006;84:1489-97.

[33] Oliveira A, Rodriguez-Artalejo F, Lopes C. The association of fruits, vegetables, antioxidant vitamins and fibre intake with high-sensitivity C-reactive protein: sex and body mass index interactions. European journal of clinical nutrition. 2009;63:1345-52.

[34] Åsgård R, Rytter E, Basu S, Abramsson-Zetterberg L, Möller L, Vessby B. High intake of fruit and vegetables is related to low oxidative stress and inflammation in a group of patients with type 2 diabetes. Scandinavian Journal of Food & Nutrition. 2007;51:149-58.

[35] Tremblay MS, Colley RC, Saunders TJ, Healy GN, Owen N. Physiological and health implications of a sedentary lifestyle. Applied physiology, nutrition, and metabolism = Physiologie appliquee, nutrition et metabolisme. 2010;35:725-40.

[36] Shivappa N, Hebert JR, Rietzschel ER, De Buyzere ML, Langlois M, Debruyne E, et al. Associations between dietary inflammatory index and inflammatory markers in the Asklepios Study. The British journal of nutrition. 2015;113:665-71.

Table 1: Participants' characteristics according to sex in Portuguese adolescents

		All [a] (n=412)			Girls [a] (n=216)			Boys [a] (n=196)			P value [b]
		(%)			***(%)***			***(%)***			
Pubertal stage (Tanner A)	2	7.2			33.3			11.7			**0.001**
	3	33.0			54.6			36.2			
	4	47.1			12.0			38.8			
	5	12.6			2.8			13.3			
Pubertal stage (Tanner B)	2	7.0			22.7			11.7			**<0.001**
	3	20.6			46.8			18.4			
	4	50.2			27.8			54.1			
	5	22.1			3.2			15.8			
Body mass index	underweight	3.6			3.7			3.6			0.574
	normal weight	66.7			65.3			68.4			
	overweight	22.1			21.8			22.4			
	obese	7.5			9.3			5.6			
Smoking habits [d]	Current smokers	1.0			0.9			1.0			**0.037**
	Occasional smokers	1.7			1.4			2.0			
	Former smokers	7.8			4.6			11.2			
	Non-smokers	89.6			93.1			85.7			
		(Median)	***(25th)***	***(75th)***	***(Median)***	***(25th)***	***(75th)***	***(Median)***	***(25th)***	***(75th)***	
Age (years)		14.9	12.6	15.7	14.9	12.6	15.6	15.0	12.7	15.8	0.143
Socio-economic status		6.0	5.0	8.0	6.0	6.0	8.0	6.0	5.0	8.0	0.416
Sedentary time [c] (minutes/day)		667.4	619.4	725.3	678.4	632.8	734.1	645.9	607.5	713.2	**0.003**
Moderate-to-vigorous physical activity [c] (minutes/day)		51.0	39.1	65.3	45.5	35.1	59.5	56.7	43.0	71.5	**<0.001**
Energy intake (kcal/day)		2 063	1 598	2 593	2 016	1 559	2 404	2 123	1 648	2 730	**0.005**

Fruit intake (portions/day)	1.2	0.72	1.92	1.28	0.71	2.00	1.18	0.75	1.82	0.735
Vegetable intake (portions/day)	0.72	0.32	1.21	0.63	0.27	1.20	0.78	0.36	1.22	0.232
Vegetable soup intake (portions/day)	0.43	0.07	0.79	0.43	0.14	0.89	0.29	0.07	0.79	**0.033**
Fruit and vegetable intake (portions/day) [e]	2.63	1.46	3.77	2.62	1.43	3.96	2.64	1.51	3.56	0.610
CRP (mg/L)	0.20	0.11	0.77	0.11	0.11	0.49	0.34	0.11	1.26	**<0.001**
IL-6 (ng/L)	1.90	1.90	3.40	1.90	1.90	3.40	1.90	1.90	3.35	0.561
C3 (mg/dL)	116.0	107.0	126.5	119.0	107.0	127.0	115.0	106.5	126.0	0.888
C4 (mg/dL)	20.0	16.0	24.0	20.0	16.0	25.0	20.0	17.0	24.0	0.561
Overall inflammatory biomarker score [f]	1.78	1.00	3.00	1.79	1.00	3.00	1.77	1.00	3.00	0.884

Abbreviations: CRP, C reactive protein; IL-6, Interleukin-6; C3, Complement component 3; C4, Complement component 4.

[a] The data shown in percentage for categorical variables and median (interquartile range) for continuous variables.

[b] P value was calculated based on Qui-squared test for categorical variables and Mann–Whitney U test for continuous variables.

[c] n=329 (55.9% girls), there is some missing values in Sedentary time and Moderate-to-vigorous physical activity variables.

[d] Qui-squared test performed with "Current smokers" and "Occasional smokers" together to improve power of test.

[e] Sum of fruit, vegetable and vegetable soup portions intake. A vegetable soup portion was considered as a vegetable portion.

[f] Overall inflammatory biomarker score were designed summing the inflammatory biomarkers (CRP, IL-6, C3 and C4) categories, wherein for each category was assigned one point if the biomarker was above the median adjusted by age and sex or zero if below the median.

Table 2: Percentage of participants according fruit and vegetable portion intake and inflammatory biomarkers categories in Portuguese adolescents

		Inflammatory Biomarkers [a]									
		CRP (mg/L)		**IL-6** (ng/L)		**C3** (mg/dL)		**C4** (mg/dL)		**Overall inflammatory biomarker score** [b]	
		lower	higher	lower	higher	lower	higher	lower	higher	0-1 (biomarkers above the median)	2-4
Fruit intake (portions/day)	<3	90.1	94.7	91.3	94.0	93.0	91.5	90.8	93.8	91.4	93.0
	≥3	9.9	5.3	8.7	6.0	7.0	8.5	9.2	6.2	8.6	7.0
Vegetable intake (portions/day)	<3	95.9	96.8	95.8	97.3	96.2	96.5	95.9	96.9	95.1	97.4
	≥3	4.1	3.2	4.2	2.7	3.8	3.5	4.1	3.1	4.9	2.6
Vegetable soup intake (portions/day)	<2	93.2	94.7	**91.6**	**98.0**	93.0	95.0	93.5	94.4	93.0	94.7
	≥2	6.8	5.3	**8.4**	**2.0**	7.0	5.0	6.5	5.6	7.0	5.3
Fruit and Vegetable intake (portions/day) [c]	<1	10.4	12.6	9.5	14.8	11.7	11.1	11.1	11.8	10.3	12.3
	1-5	76.6	78.9	77.9	77.2	78.4	76.9	75.1	80.5	76.2	78.9
	≥5	13.1	8.4	12.5	8.1	9.9	12.1	13.8	7.7	13.5	8.8

Abbreviations: CRP, C reactive protein; IL-6, Interleukin-6; C3, Complement component 3; C4, Complement component 4.

[a] The data shows column percentage by each inflammatory biomarker categories. A p value was calculated based on Qui-squared test. Differences statically significant are shown in bold.

[b] Overall inflammatory biomarker score were designed summing the inflammatory biomarkers (CRP, IL-6, C3 and C4) categories, wherein for each category was assigned one point if the biomarker was above the median adjusted by age and sex or zero if below the median.

[c] Sum of fruit, vegetable and vegetable soup portions intake.

* p=0.009

Table 3. Association between fruit, vegetable, vegetable soup intake and inflammatory biomarkers among Portuguese adolescents

	Fruit intake OR (95% CI) <3portions (ref.: ≥3portions)	**Vegetable intake** OR (95% CI) <3portions (ref.: ≥3portions)	**Vegetable soup intake** OR (95% CI) <2portions (ref.: ≥2portions)
CRP models			
Model 1 [a]	1.06 (0.66-1.69)	1.30 (0.45-3.71)	1.30 (0.57-2.98)
Model 2 [b]	1.04 (0.65-1.66)	1.29 (0.45-3.71)	1.20 (0.81-1.76)
Model 3 [c]	1.31 (0.70-2.48)	2.13 (0.53-8.54)	2.20 (0.73-6.63)
Model 4 [d]	0.85 (0.64-1.13)	1.98 (0.46-8.50)	1.98 (0.65-6.02)
IL-6 models			
Model 1 [a]	1.06 (0.65-1.72)	1.58 (0.50-5.06)	**4.44 (1.31-15.10)***
Model 2 [b]	1.08 (0.66-1.76)	1.59 (0.50-5.08)	**4.49 (1.32-15.29)***
Model 3 [c]	1.51 (0.78-2.91)	1.85 (0.36-9.56)	**13.73 (1.70-105.43)***
Model 4 [d]	1.03 (0.77-1.37)	1.14 (0.19-7.03)	**12.95 (1.63-102.84)***
C3 models			
Model 1 [a]	0.71 (0.45-1.13)	1.07 (0.38-3.01)	1.43 (0.63-3.27)
Model 2 [b]	0.71 (0.45-1.14)	1.07 (0.38-3.01)	1.44 (0.63-3.28)
Model 3 [c]	1.07 (0.56-2.07)	1.60 (0.39-6.60)	2.63 (0.85-8.08)
Model 4 [d]	0.92 (0.70-1.21)	1.37 (0.33-5.74)	2.51 (0.82-7.71)
C4 models			
Model 1 [a]	1.19 (0.75-1.90)	1.36 (0.48-3.90)	1.15 (0.51-2.61)
Model 2 [b]	1.19 (0.74-1.91)	1.36 (0.48-3.90)	1.15 (0.51-2.60)
Model 3 [c]	1.52 (0.82-2.80)	2.62 (0.62-11.02)	1.45 (0.53-3.96)
Model 4 [d]	0.88 (0.67-1.15)	1.92 (0.44-8.30)	1.32 (0.48-3.64)
Overall inflammatory biomarker score models [e]			
Model 1 [a]	0.95 (0.60-1.52)	1.88 (0.66-5.39)	1.35 (0.60-3.04)
Model 2 [b]	0.95 (0.60-1.53)	1.88 (0.66-5.40)	1.36 (0.60-3.05)
Model 3 [c]	1.43 (0.75-2.73)	3.16 (0.76-13.18)	2.37 (0.80-7.02)
Model 4 [d]	1.17 (0.59-2.30)	2.46 (0.56-10.31)	2.11 (0.71-6.33)

Abbreviations: OR, odds ratio; CI, confidence interval; CRP, C reactive protein; IL-6, Interleukin-6; C3,

Complement component 3; C4, Complement component 4.

[a] All models 1 were crude models;

[b] All models 2 were adjusted for sex;

[c] All models 3 were adjusted for sex, age, pubertal stage – Tanner A and B, body mass index, energy intake, socio-economic status, sedentary time, moderate-to-vigorous physical activity, and smoking habits. Model were performed with n=329 (55.9% girls) because there is some missing values in Sedentary time and MVPA variables.

[d] Models 4 were adjusted for same variables from models 3 plus the others food group intake as covariates; on fruit portion intake models we also included vegetable portion intake and vegetable soup portion intake; on vegetable portion intake models we also included fruit portion intake and vegetable soup portion intake; and, on vegetable soup portion intake models we also included fruit portion intake and vegetable portion intake. Models were performed with n=329 (55.9% girls) because there is some missing values in Sedentary time and MVPA variables.

[e] Overall inflammatory biomarker score were designed summing the inflammatory biomarkers (CRP, IL-6, C3 and C4) categories, wherein for each category was assigned one point if the biomarker was above the median adjusted by age and sex or zero if below the median.

* $p<0.05$; ** $p<0.01$.

Table 4: Association between five or more daily fruit and vegetable intake on inflammatory biomarkers among Portuguese adolescents

	Fruit and vegetable portions OR (95% CI)			
	≥5 portions/day	≥1 to <5 portions/day	<1 portion/day	p for trend [a]
CRP Models				
Model 1 [b]	1.00	1.60 (0.84-3.06)	1.89 (0.82-4.37)	0.114
Model 2 [c]	1.00	1.58 (0.83-3.03)	1.88 (0.81-4.34)	0.120
Model 3 [d]	**1.00**	**2.90 (2.90-6.86)****	**5.24 (1.62-16.97)***	**0.005**
IL-6 models				
Model 1 [b]	1.00	1.61 (0.80-3.23)	**2.42 (1.01-5.80)***	0.059
Model 2 [c]	1.00	1.57 (0.78-3.15)	**2.45 (1.02-5.88)***	0.056
Model 3 [d]	**1.00**	2.40 (0.96-6.00)	**5.36 (1.64-17.53)***	**0.008**
C3 models				
Model 1 [b]	1.00	0.80 (0.43-1.50)	0.77 (0.34-1.75)	0.485
Model 2 [c]	1.00	0.81 (0.43-1.51)	0.77 (0.34-1.75)	0.491
Model 3 [d]	1.00	1.92 (0.81-4.53)	2.94 (0.90-9.67)	0.072
C4 models				
Model 1 [b]	1.00	1.93 (1.00-3.72)	1.92 (0.83-4.46)	0.077
Model 2 [c]	1.00	1.93 (1.00-3.72)	1.92 (0.83-4.46)	0.077
Model 3 [d]	**1.00**	**2.78 (1.22-6.33)***	**3.39 (1.12-10.28)***	**0.016**
Overall inflammatory biomarker score models [e]				
Model 1 [b]	1.00	1.59 (0.85-2.97)	1.84 (0.81-4.22)	0.118
Model 2 [c]	1.00	1.60 (0.85-2.99)	1.85 (0.81-4.23)	0.116
Model 3 [d]	**1.00**	**4.15 (1.73-9.97)****	**8.14 (2.44-27.18)****	**<0.001**

Abbreviations: OR, odds ratio; CI, confidence interval; CRP, C reactive protein; IL-6, Interleukin-6; C3, Complement component 3; C4, Complement component 4.

[a] Linear trends were tested for significance by assigning each subjects the median frequency of fruit and vegetable intake (portion/day) for the categories and treating this value as a continuous variable.

[b] All models 1 were crude models;

[c] All models 2 were adjusted for sex;

[d] All models 3 were adjusted for sex, age, pubertal stage – Tanner A and B, body mass index, energy intake, socio-economic status, sedentary time, moderate-to-vigorous physical activity, and smoking habits. Model were performed with n=329 (55.9% girls) because there is some missing values in Sedentary time and MVPA variables.

[e] Overall inflammatory biomarker score were designed summing the inflammatory biomarkers (CRP, IL-6, C3 and C4) categories, wherein for each category was assigned one point if the biomarker was above the median adjusted by age and sex or zero if below the median.

* $p<0.05$; ** $p<0.01$.

7.3. PAPER III

Associations between fruit and vegetable variety and low-grade inflammation in Portuguese adolescents from LabMed physical activity study.

[Submitted for publication]

Associations between Fruit and Vegetable Variety and Low-Grade Inflammation in Portuguese Adolescents from LabMed Physical Activity Study

Juliana Almeida-de-Souza[1,2], Rute Santos[3,4,5], Luis Lopes[3], Sandra Abreu[3], Carla Moreira[3], Patrícia Padrão[2,6], Jorge Mota[3], Pedro Moreira[2,3,6].

[1]School of Health, Polytechnic Institute of Bragança, Portugal.

[2]Faculty of Nutrition and Food Sciences, University of Porto, Portugal.

[3]Research Centre in Physical Activity, Health and Leisure, Faculty of Sport, University of Porto, Portugal.

[4]Early Start Research Institute, University of Wollongong, Australia.

[5]University Institute of Maia. Portugal.

[6]Institute of Public Health, University of Porto, Portugal

Correspondence to:

Juliana Almeida-de-Souza. School of Health, Polytechnic Institute of Bragança. Av. D. Afonso V – 5300-121 Bragança, Portugal. Telephone number: 273 330 950; Fax number: 273 327 915; e-mail: julianaalmeida@ipb.pt

Acknowledgments:

The authors gratefully acknowledged the participation of all adolescents and their parents, teachers and schools of the LabMed and Physical Activity Study. They also acknowledge the cooperation of volunteer's subjects, the Department of Hygiene and Epidemiology from Medical School (University of Porto) for the conversion the food frequency questionnaire data into nutrients, and the Research Centre in Physical Activity, Health and Leisure (University of Porto) for the sponsoring the LabMed and Physical Activity Study. The research Centre in Physical Activity, Health and Leisure (University

of Porto) is supported by the Portuguese Foundation for Science and Technology (UID/DTP/00617/2013). Almeida-de-Souza J had been supported by program for advanced training (SFRH/PROTEC/68092/2010). Rute Santos has a Discovery Early Career Research Award from the Australian Research Council (DE150101921).

Abstract

Purpose: The dietary guidelines for the consumption of a variety of fruits and vegetables have been recognized as an important factor for achieving healthy eating patterns to reduce the risk of chronic disease throughout the lifespan. Our aim is to assess the association between fruit and vegetable variety and low-grade inflammation in adolescents.

Methods: This cross-sectional analysis was conducted with 412 adolescents (ages 14.4±1.7years; 52%girls). The consumption of a variety of fruits and vegetables was assessed with a food-frequency questionnaire, considering the number of individual/category of fruit or vegetable intake at least once month, and categorized into tertiles. Blood samples were collected to determine C-reactive protein (CRP), interleukin-6 (IL-6), complement component 3 (C3), and 4 (C4). We created categories of lower or higher (inflammatory state) for each biomarker, considering sex- and age-adjusted median values. We computed an overall inflammatory biomarker overall based on biomarker categories, and created categories of 0-1 or 2-4 biomarkers above the median. The odds ratio (OR) and 95% interval confidence (95%CI) were calculated from binary logistic regression to estimate the magnitude of association between fruit and vegetable variety and inflammatory biomarkers.

Results: Adolescents with a greater variety of vegetable consumption (≥13categories/month) had lower odds of having a higher CRP (OR=0.31, 95%CI=0.15-0.64, p_{trend}=0.004) when compared to those with lower variety consumption (≤6categories/month), independent of vegetable quantity intake.

Conclusions: The consumption of a variety of vegetables is inversely associated with lower CRP. This finding supports the current dietary guidelines regarding the consumption of a variety of vegetables.

Keywords:

C-reactive protein, Interleukin 6, Complement C3, Complement C4, Inflammatory biomarker score, Variety of diet.

Introduction

Many chronic conditions have been associated with low-grade inflammation [1-4] such as obesity [5], type 2 diabetes [5,6], cardiovascular diseases [7,8], and cancer [9,10], in adulthood and seniors. It has also been reported that low-grade inflammation in children and adolescents is associated with obesity [11,12], central obesity [13,14], metabolic syndrome [15,16], impaired endothelial function and atherosclerosis [17]. Low-grade inflammation is characterized by the increased concentration of inflammatory biomarkers in the bloodstream [2] and several biomarkers including cytokines and acute phase proteins have been used to define low-grade inflammation. C-reactive protein (CRP) is the most widely used indicator of low-grade inflammation; however, its diagnostic criteria have not been precisely defined [1,3].

Epidemiological evidence shows that fruit and vegetable intake is inversely associated with low-grade inflammation [1]. Studies have reported the importance of increased quantities of fruit and vegetable intake, but little is known about the role of fruit or vegetable variety. However, several dietary guidelines recommend a varied diet, emphasizing plant-based foods in particular [18]. The World Health Organization is very objective in its guidelines and recommends eating “a variety of vegetables and fruits” [19]. More recently, the U.S. updated its dietary guidelines to separate fruit and vegetable groups, recommending “a variety of vegetables” beyond a variety of nutrient-dense foods [20].

The contribution to disease prevention of a large variety of fruit and vegetable intake is limited and results are inconclusive. Several studies have found no relationship between fruit and vegetable variety and incidence of cardiovascular disease [21,22], stroke [22], or bladder cancer [23], whereas other studies reported negative associations with the risk of type 2 diabetes [24], and cancer [25,26].

A dramatic increase in chronic disease closely connected with low-grade inflammation has been observed in youth [27,28], and these conditions can be associated with complications in adulthood, including early onset cardiovascular disease [28]. However, an increased intake of fruits and vegetables can reduce the incidence of chronic diseases, especially cardiovascular disease [29,30]. Fruit and vegetable intake among adolescents is low [31,32], even lower than in adults [33], and does not reflect the desired vegetable variety recommended in dietary guidelines [34]. Our aim is to study the association of fruit and vegetable variety with low-grade inflammation in adolescents, as measured by four inflammatory biomarkers and one overall score.

Subjects and Methods

Study design and sampling

This is a cross-sectional analysis from the baseline (fall 2011) of the study titled Longitudinal Analysis of Biomarkers and Environmental Determinants of Physical Activity Study (LabMed Physical Activity Study), a school-based prospective cohort study conducted in five schools in the north of Portugal that have collaborative agreements previously established with our research centre. A full description of the study protocol can be found elsewhere [35,36].

Briefly, a previous sample size was estimated at 1086 subjects, considering a prevalence of 14% for the combined healthy diet/physical activity pattern exposure [40], for a power of 0.80 and two-tailed significance of 5%, and an expected dropout rate of 20%. Students from grades seven and ten were invited to participate (N=1678), and the criterion for participation was that both the adolescents and their parents or legal guardian provided written informed consent (n=1229). However, blood samples were collected only in subjects without known disease. All data were collected in schools.

For this cross-sectional study, the sample comprises 412 adolescents (216 girls), between 12 and 18 years old, for whom both blood sample collection (n=534) and accurate dietary intake (n=622) were available.

Ethical standards

All ethical issues have been guaranteed, and the study was conducted in accordance with the World Medical Association's Helsinki Declaration for Human Studies. Written informed consent was obtained from adolescents and their parents or guardians. The Portuguese Data Protection Authority (#1112434/2011), the Portuguese Ministry of Science and Education (0246200001/2011) and the Faculty of Sport, University of Porto, approved the LabMed Physical Activity Study.

Inflammatory biomarkers assessment and overall inflammatory biomarker score

After at least ten hours of fasting, participants in a sitting position donated a blood sample collected from the antecubital vein. The samples were refrigerated (4º– 8°C) and sent to a laboratory to determine the inflammatory biomarkers: CRP by the latex-enhanced turbidimetric assay (Siemens

Advia 1600/1800, Erlangen, Germany); Interleukin-6 (IL-6) by the chemiluminescence immunoassay (Immulite 2000, Diagnostic Products Corporation, Los Angeles, CA); and Complement component 3 (C3) and 4 (C4) by the immunoturbidimetric assay (Siemens Advia 1600/1800, Erlangen, German).

An elevation in the concentration of inflammatory biomarkers can be minimal or absent in low-grade inflammation [10], and no accepted international cut-points have been defined for adolescents. Therefore, we have decided to consider the inflammatory biomarkers' sex- and age-adjusted median values in order to create two groups of participants: higher or lower (inflammatory state). The final median values for each category (higher/lower) were 0.11/0.92 mg/L for CRP, 1.90/4.20 ng/L for IL-6, 107.00/127.00 mg/dL for C3, and 17.00/25.55 mg/dL for C4.

We have also calculated an overall inflammatory biomarker score, assigning one point to subjects who were above the sex- and age-adjusted medians and zero to those who were below, for each inflammatory biomarker and totaled all points assigned. The overall inflammatory biomarker score varies from zero to four points. Two categories were defined: 0–1 (49.9%) and 2–4 (50.1%) inflammatory biomarkers above the median.

Physical activity and sedentary time assessment

GT1M Actigraph accelerometers (ActiGraph, Pensacola, Florida, USA) were used to assess physical activity and sedentary time. This is a lightweight, biaxial monitor that adolescents wore attached tightly at the hip on the right side of the body with the notch facing upwards. It was used during all waking hours and removed during water-based activities for five consecutive days (three weekdays and two weekend days). The epoch length was set to 2 seconds to allow a more detailed estimate of physical activity intensity.

An automated data-reduction program (ActivLive 6.12, ActiGraph, Pensacola, Florida, USA) was utilized to analyze accelerometer data from individual participants. Non-wearing time was considered when 60 minutes of consecutive zeros were flagged. A valid day was considered to consist of at least 8 hours of accelerometer use. The participants had to have at least three valid days to be included (two weekdays and one weekend day).

After screening, the cut-points proposed by Evensons et al. [37] were used to determine physical activity intensities according to the raw activity *counts*. Physical activity was expressed in mean

142 counts.min^{-1} and also in estimates of the time spent in moderate-to-vigorous physical activity and
143 sedentary time, using minutes.day^{-1}.

144

145 **Dietary intake assessment**

146 A self-administered, semi-quantitative food-frequency questionnaire validated for a Portuguese
147 population [38] and adapted to adolescents [39] was used to assess dietary intake in the previous 12
148 months. The food-frequency questionnaire lists 91 food and beverage items or categories with a
149 standard portion size, nine response options (from *never or less than once per month* to *six or more*
150 *times per day*), and a seasonal alternative. Blank lines were included for participants to add any food
151 that was not listed. The portion size in grams was multiplied by the multiple/fraction of daily frequency
152 intake and by a seasonality variation factor for each option selected, and dietary intake was estimated.
153 The Food Processor Plus program version SQL (ESHA Research, Salem, OR, USA), supplemented
154 with the Portuguese food-composition databases [40,41], was used to convert food to energy and
155 nutrient intakes.
156 We included only accurate food-frequency questionnaires, using the Goldberg cut-off method [42]
157 adapted by Black [43], for the determination and exclusion of dietary assessment misreporting. Thus,
158 we calculated the basal metabolic rate using the Schofield equation [44], considering sex and age, and a
159 ratio energy intake/basal metabolic rate to compare with 95% confidence limits (cut-offs). The cut-offs
160 for our sample were determined using the following: mean physical activity level, number of days of
161 dietary assessment, within-subject coefficient of variation in energy intake, between-subject variation
162 in physical activity, and variation in basal metabolic rate. The mean physical activity level was 1.23,
163 calculated using accelerometer data (counts.minutes^{-1} and daily use time) per the Trost equation [45].
164 We considered 21 days of dietary assessment, according to Black, for the food-frequency questionnaire
165 [43]. The within-subject coefficient of variation in energy intake was calculated considering mean and
166 standard deviation of energy intake in our sample and the number of dietary assessments. The between-
167 subject variation in physical activity was calculated considering the mean and standard deviation of
168 physical activity level in our sample. A figure of 8.5% was used for the coefficient of variation of
169 repeated basal metabolic rate measurements, as Black suggested [43]. The cut-offs achieved were 0.61
170 and 2.48; therefore, adolescents with energy intake/basal metabolic rate outside of this range were

considered to have misreported dietary assessment, and 150 adolescents were excluded from the statistical analysis.

The variety of intake of fruits and vegetables was defined by scores considering the total number of unique individual/categories of fruits or vegetables consumed at least once per month over the past 12 months; no point was attributed for consumption < *once per month*, while 1 point was given for an intake of ≥ *1 time per month*. For the fruit variety, the score considered a maximum of 12 categories, as follows: apple/pear, orange/tangerine, banana, kiwi, strawberry, cherry, peach/plum, melon/watermelon, persimmon, fig/medlar/apricot, grape, and papaya/mango; it excluded candied fruit and fruit juices because their sugar content is unknown and also to avoid replicating the same fruit variety listed above. For the vegetable variety, the score considered a maximum of 15 categories, as follows: white/savoy cabbages, bunch/Portuguese cabbages, curly kale, broccoli, cauliflower/Brussel sprout, turnip sprout/turnip greens/spinach, green bean, lettuce/watercress, onion, carrot, turnip, tomato, pepper, cucumber, pea/broad bean; it excluded starchy vegetables like potatoes and dried pulses because their starch content is very different from other foods and because there is no consensus about the inclusion of these foods in the vegetable category [46,47].

Adolescents were classified as having low-, medium-, and high-variety intake, according to the tertiles of the fruit and vegetable variety scores, considering the following: 1st tertile ≤ 6 categories/month, 2nd tertile 7–12 categories/month, and 3rd tertile ≥ 13 categories/month for vegetable variety; and for fruit variety, 1st tertile ≤ 9 categories/month, 2nd tertile 10–11 categories/month, and 3rd tertile = 12 categories/month.

Anthropometric data

Weight and height were measured with a scale (TANITA Inner Scan BC532, Tokyo, Japan) and a stadiometer (SECA 213, Hamburg, Germany) respectively, with the adolescent standing upright, lightly dressed, and without shoes. We calculated the body mass index from the ratio of weight (kg) to height squared (m2), and adolescents were classified according to Cole's body mass index categories [48].

Pubertal stage

Adolescents self-reported their pubertal stage (from 1 to 5) relatively to secondary sex characteristics,

according to the criteria of Tanner and Whitehouse [49]. Briefly, Tanner A indicates the stage of breast development in girls and genitalia development (penis size and testicular volume) in boys; and Tanner B indicates the stage of public hair distribution in both sexes.

Socio-economic status

Socioeconomic status was measured by the family affluence scale [50], from 0 to 9 points; a lower score indicates a lower socioeconomic status.

Smoking habits

Adolescents self-reported their smoking habits and were classified according to the World Health Organization's criteria [51] as: non-smokers, former smokers (individuals who had stopped smoking for at least 6 months), occasional smokers (individuals who smoked, on average, less than one cigarette a day), and current smokers (individuals who smoked at least one cigarette a day).

Statistical Analyses

Participants' characteristics are presented for the whole sample and by sex as percentages for categorical variables and as medians and 25th and 75th percentiles for continuous variables. To assess the sex differences for each characteristic, the Mann-Whitney U test was used for continuous variables and a Chi-square test for categorical variables.

We performed a Chi-square test to study the difference between inflammatory biomarker categories across the variety of fruit and vegetable intake (in tertiles).

To study the association between fruit and vegetable variety tertiles and inflammatory biomarker categories, we constructed binary logistic regression models. There were four models for each inflammatory biomarker and for the overall inflammation biomarker score, as dependent variables, and fruit or vegetable variety tertiles as predictors, using the first variety tertiles as reference: crude models, and adjusted models 1, 2 and 3. Models 1 were sex-adjusted. Models 2 were adjusted for sex, age, pubertal stage (Tanner A and B), body mass index, energy intake, socioeconomic status, sedentary time, moderate-to-vigorous physical activity, and smoking habits. Models 3 were adjusted for the same

variables as models 2 plus for the quantity of fruit on the fruit variety model or the quantity of vegetable on the vegetable variety model. A multicollinearity diagnosis between independent variables was conducted and nothing was detected.

Post hoc power calculations were conducted considering the smaller sample size (n=329), the smaller odds ratio for fully adjusted model (OR=0.3), a null hypothesis value of 0.5, a significance level of 0.05, and we achieved a power of >0.99.

A 0.05 level of significance and 95% CI (confidence interval) were considered. Data analysis was performed using the statistical package SPSS®, version 21.0 (SPSS Inc., Chicago, IL, USA), and power analysis was performed using G*Power, version 3.1 (Faul, Erdfelder, Lang, & Buchner, 2007).

Results

The sample characteristics are presented in Table 1. On average, boys presented a higher variety of fruit and vegetable intake and higher levels of CRP than girls ($p<0.05$ for both).

As depicted in Table 2, adolescents within the 3rd tertile of vegetable variety (higher variety) had a higher prevalence of a lower level of CRP, IL-6, C3, and the overall inflammatory biomarker score than adolescents within the 1st or 2nd tertiles. In respect to fruit variety, no differences are observed.

As depicted in Table 3, adolescents with higher vegetable variety intake (3rd tertile) had a lower prevalence of a higher level of CRP (OR=0.31; 95%CI:0.15-0.64; p_{trend}=0.004) when compared to adolescents with lower variety (1st tertile) in the fully adjusted model (including vegetable quantity intake). In addition, adolescents with higher vegetable variety intake (3rd tertile) had a lower prevalence of higher overall inflammatory biomarker score (OR=0.47; 95%CI:0.25-0.87; p_{trend}=0.044) when compared to adolescents with lower variety (1st tertile) in the model adjusted for biological and lifestyle variables. However, this association is lost when we include vegetable quantity intake. In respect to fruit variety, C4 presented a positive statistically significant trend. However, the third tertile was not statistically significant.

Discussion

The main finding of the present study emphasizes the importance of consuming a variety of vegetables to reduce low-grade inflammation, considering that high vegetable variety is inversely associated with CRP (p for trend <0.01, in all models). This relationship remains independent of vegetable quantity intake (p for trend =0.002 in the fully adjusted model, including vegetable quantity). These results support the current dietary guidelines regarding the importance of vegetable variety for a healthy eating pattern [20].

The consumption of vegetables and fruits has been reported to be an important factor for the prevention of low-grade inflammation, but the focus of the discussion has especially been on the quantity of fruit and vegetable intake, while little has been discussed regarding variety, particularly for each of these two food groups [1]. Consuming a diet characterized by a large variety of vegetables, rather than consuming a monotonous diet, may favor reduced exposure to any undesirable, harmful components [52] and enables individuals to achieve intake of a larger number of nutrients and bio-active components [19,53] including vitamins, minerals, and phytochemicals [54]. In fact, plant-based foods are recognized as rich in bioactive phytochemicals [55], including those that have been widely studied such as flavonoids and carotenoids, and other unknown bioactive components that could be beneficial, either alone or in combination [54]. Those bioactive compounds are recognized as having an anti-inflammatory effect, although their mechanisms remain poorly understood [56]; thereforethe, the exact components and their dosages for supplementation purposes to reduce inflammation have not yet been identified [57]. Unlike supplementation, the food matrix is accompanied by a variety of substances that can exert additive and synergistic functions [57], so the beneficial effects of those bioactive components and their diversity can also be attributed to additive and synergistic effects responsible for their potent antioxidant activities [58]. Despite our cross-sectional design, our results seem to reflect the additive and synergistic effects of the combination of different bioactive compounds, in a variety of vegetables as whole foods as an import factor in reducing low-grade inflammation, in this study assessed by the inflammatory biomarker CRP.

It is interesting to note that this inverse relationship between vegetable variety intake and low-grade inflammation was found only for CRP, though the overall inflammatory biomarker score models were also inverse associated with vegetable variety intake, but this association is lost when adjusted for

vegetable quantity intake. The evidence indicates that certain bioactive compounds can be helpful in decreasing cardiovascular disease risk factors [59], and CRP is recognized as an established inflammatory biomarker for the risk of cardiovascular events [60]. Thus, our findings are in accordance with a review showing that a number of dietary intervention studies have provided evidence that dietary flavonoids can modulate CRP production [3]. In addition, studies have indicated that total flavonoid intake[61] or serum level of β-carotene[62] are inversely associated with serum CRP.

Our results show a significant association (p for trend < 0.05) between low-grade inflammation and vegetable variety but not for fruit variety, contrary to what we expected. Furthermore, intermediate (2nd tertile) and higher (3rd tertile) fruit variety scores were positively and significantly associated with C4 (in all models) and IL-6 (in model 3), respectively, which remains to be explained. Several studies have found an inverse association between fruit quantity and inflammatory biomarkers, especially CRP [63-66], and IL-6 [67]. However, the role of C3 and C4 remains unknown. According to our knowledge, no studies have been published about fruit variety and low-grade inflammation; when fruit and vegetable variety are measured together, the negative association with CRP may be described [68]. However, considering that the composition of fruits and vegetables may be different among them, particularly for sugar content (fructose) [52], to consider their separate effects seems to be relevant. One factor that could be addressed in future research is the focus on the fructose content of fruit. A direct association between fructose intake and insulin resistance may exist [69,70], and it has been found that beverages sweetened with fructose can potentially increase CRP [71]. In fact, high fructose intake may be an inductor form of visceral adipose increase, which may stimulate inflammatory responses that further promote liver lipid accumulation and impair hepatic insulin signalling.[72] Furthermore, a positive relation between C4 and impaired glucose metabolism in adolescents were already observed.[73] Finds about IL-6 are controversial, while some authors have shown a positive relationship with impaired glucose metabolism in adolescents,[74] other authors have demonstrated no association.[73]

Furthermore, we adjusted our models for important covariates of low-grade inflammation, such as physical activity, sedentary time, and pubertal stage, which may also help to explain the lack of significant associations in our study. In an experimental study, Raynor and Osterholt demonstrated that wide fruit variety (different fruit snacks) increases the amount of fruit consumed compared to fruit monotony (same fruit snack) [75], and in our study, we show a higher mean consumption of fruit

quantity (230.0 ± 178.2g) and variety (9.9 ± 2.9 categories in a maximum of 12) related to vegetable quantity (143.9 ± 150.8g) and variety (9.1 ± 4.6 categories in a maximum of 15). We also observed that fruit variety is more homogeneous (lower standard deviation) than vegetable variety, and therefore, vegetable variety was more discriminant than fruit variety on showing low-grade inflammation.

Several limitations should be acknowledged. First, variety intake has been recognized as a factor for increased quantity intake [76]. However, in model adjusted 3, we included the quantity intake, and, in fact, the results changed in overall inflammatory biomarker score model. Second, we used a food-frequency questionnaire that combines several fruits and vegetables into single items, making it impossible to distinguish one from another, and thus the variety may have possibly been underestimated. Furthermore, several studies used food-frequency questionnaire to measure fruit and vegetable variety intake [21,23,26,68], which may be a positive factor for comparison studies. Third, because of the lack of established cut-off points for inflammatory biomarkers for adolescents, and because the increase of inflammatory biomarkers in low-grade inflammation may be very low or even absent [2], we used sex-age-adjusted median values to create two categories of inflammatory states, permitting us to rank the sample and classify it according to lowest and highest inflammatory state. Nevertheless, our cut-off points for CRP (varying from 0.11 to 0.79mg/dL) are close to that reported by Visser et al (0.22 mg/dL) [12], reporting a positive association with overweight in children and adolescents. Forth, in addition to food consumption, also confounding factors were based on self-reports, namely pubertal stage, which always gives a chance of bias.

The strengths of our work include, beyond the novelty of its aim, the use of objectively measured physical activity and sedentary time as covariates, once sedentary time is considered a risk factor for cardiovascular health independent of physical activity levels [77], and physical activity has been reported as an important inductor of an anti-inflammatory environment [78]. Moreover, we used only accurate food-frequency questionnaires, according to Goldberg's method [43], which is useful for evaluating the mean population bias in reports of energy intake. It recommends the use of information on physical activity rather than theoretical values available, which we followed. Furthermore, our models considered other important potential confounders such as age, body mass index, sex, and smoking habits that have been shown to be associated with inflammatory biomarker concentration [2,3]. Another strength is the use of a set of inflammatory biomarkers and an overall inflammatory

biomarker score because inflammatory biomarkers, in general and in healthy people, are non-specific pro-inflammatory response markers [3].

In summary, vegetable variety intake, independent of vegetable quantity intake, was inversely associated with CRP after adjustments for several biological and lifestyle confounders. These findings support the recommendation for choosing a variety of vegetables to ensure a healthy eating pattern. Variety related to fruit intake remained unclear. Future studies using longitudinal methodologies are necessary to confirm or rule out our findings.

Conflict of interest

The authors declare that they have no conflict of interest.

References

1. Calder PC, Ahluwalia N, Brouns F, Buetler T, Clement K, Cunningham K, Esposito K, Jonsson LS, Kolb H, Lansink M, Marcos A, Margioris A, Matusheski N, Nordmann H, O'Brien J, Pugliese G, Rizkalla S, Schalkwijk C, Tuomilehto J, Warnberg J, Watzl B, Winklhofer-Roob BM (2011) Dietary factors and low-grade inflammation in relation to overweight and obesity. The British journal of nutrition 106 Suppl 3:S5-78. doi:10.1017/s0007114511005460

2. Calder PC, Ahluwalia N, Albers R, Bosco N, Bourdet-Sicard R, Haller D, Holgate ST, Jonsson LS, Latulippe ME, Marcos A, Moreines J, M'Rini C, Muller M, Pawelec G, van Neerven RJ, Watzl B, Zhao J (2013) A consideration of biomarkers to be used for evaluation of inflammation in human nutritional studies. The British journal of nutrition 109 Suppl 1:S1-34. doi:10.1017/s0007114512005119

3. Minihane AM, Vinoy S, Russell WR, Baka A, Roche HM, Tuohy KM, Teeling JL, Blaak EE, Fenech M, Vauzour D, McArdle HJ, Kremer BH, Sterkman L, Vafeiadou K, Benedetti MM, Williams CM, Calder PC (2015) Low-grade inflammation, diet composition and health: current research evidence and its translation. The British journal of nutrition 114 (7):999-1012. doi:10.1017/s0007114515002093

4. Pawelec G, Goldeck D, Derhovanessian E (2014) Inflammation, ageing and chronic disease. Current opinion in immunology 29:23-28. doi:10.1016/j.coi.2014.03.007

5. van Greevenbroek MM, Schalkwijk CG, Stehouwer CD (2013) Obesity-associated low-grade inflammation in type 2 diabetes mellitus: causes and consequences. The Netherlands journal of medicine 71 (4):174-187

6. Spranger J, Kroke A, Mohlig M, Hoffmann K, Bergmann MM, Ristow M, Boeing H, Pfeiffer AF (2003) Inflammatory cytokines and the risk to develop type 2 diabetes: results of the prospective population-based European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam Study. Diabetes 52 (3):812-817

7. Berg AH, Scherer PE (2005) Adipose tissue, inflammation, and cardiovascular disease. Circulation research 96 (9):939-949. doi:10.1161/01.res.0000163635.62927.34

8. Libby P (2012) History of Discovery: Inflammation in Atherosclerosis. Arteriosclerosis, thrombosis, and vascular biology 32 (9):2045-2051. doi:10.1161/ATVBAHA.108.179705

9. Fernandes JV, Cobucci RN, Jatoba CA, Fernandes TA, de Azevedo JW, de Araujo JM (2015) The role of the mediators of inflammation in cancer development. Pathology oncology research : POR 21 (3):527-534. doi:10.1007/s12253-015-9913-z

10. Ostan R, Lanzarini C, Pini E, Scurti M, Vianello D, Bertarelli C, Fabbri C, Izzi M, Palmas G, Biondi F, Martucci M, Bellavista E, Salvioli S, Capri M, Franceschi C, Santoro A (2015) Inflammaging and cancer: a challenge for the Mediterranean diet. Nutrients 7 (4):2589-2621. doi:10.3390/nu7042589

11. Ford ES, Galuska DA, Gillespie C, Will JC, Giles WH, Dietz WH (2001) C-reactive protein and body mass index in children: findings from the Third National Health and Nutrition Examination Survey, 1988-1994. The Journal of pediatrics 138 (4):486-492. doi:10.1067/mpd.2001.112898

12. Visser M, Bouter LM, McQuillan GM, Wener MH, Harris TB (2001) Low-grade systemic inflammation in overweight children. Pediatrics 107 (1):E13

13. Galcheva SV, Iotova VM, Yotov YT, Bernasconi S, Street ME (2011) Circulating proinflammatory peptides related to abdominal adiposity and cardiometabolic risk factors in healthy

prepubertal children. European journal of endocrinology / European Federation of Endocrine Societies 164 (4):553-558. doi:10.1530/eje-10-1124

14. Warnberg J, Nova E, Moreno LA, Romeo J, Mesana MI, Ruiz JR, Ortega FB, Sjostrom M, Bueno M, Marcos A (2006) Inflammatory proteins are related to total and abdominal adiposity in a healthy adolescent population: the AVENA Study. The American journal of clinical nutrition 84 (3):505-512

15. Ford ES, Ajani UA, Mokdad AH (2005) The metabolic syndrome and concentrations of C-reactive protein among U.S. youth. Diabetes care 28 (4):878-881

16. Lambert M, Delvin EE, Paradis G, O'Loughlin J, Hanley JA, Levy E (2004) C-reactive protein and features of the metabolic syndrome in a population-based sample of children and adolescents. Clinical chemistry 50 (10):1762-1768. doi:10.1373/clinchem.2004.036418

17. Jarvisalo MJ, Harmoinen A, Hakanen M, Paakkunainen U, Viikari J, Hartiala J, Lehtimaki T, Simell O, Raitakari OT (2002) Elevated serum C-reactive protein levels and early arterial changes in healthy children. Arteriosclerosis, thrombosis, and vascular biology 22 (8):1323-1328

18. Montagnese C, Santarpia L, Buonifacio M, Nardelli A, Caldara AR, Silvestri E, Contaldo F, Pasanisi F (2015) European food-based dietary guidelines: a comparison and update. Nutrition (Burbank, Los Angeles County, Calif) 31 (7-8):908-915. doi:10.1016/j.nut.2015.01.002

19. World Health Organization (2003) Food based dietary guidelines in the WHO European Region. Copenhagen, Denmark

20. U.S. Department of Health and Human Services, and U.S. Department of Agriculture (2015) 2015–2020 Dietary Guidelines for Americans. 8th edn. US Government Printing Office Washington, DC,

21. Bhupathiraju SN, Wedick NM, Pan A, Manson JE, Rexrode KM, Willett WC, Rimm EB, Hu FB (2013) Quantity and variety in fruit and vegetable intake and risk of coronary heart disease. The American journal of clinical nutrition 98 (6):1514-1523. doi:10.3945/ajcn.113.066381

22. Oude Griep LM, Verschuren WM, Kromhout D, Ocke MC, Geleijnse JM (2012) Variety in fruit and vegetable consumption and 10-year incidence of CHD and stroke. Public health nutrition 15 (12):2280-2286. doi:10.1017/s1368980012000912

23. Buchner FL, Bueno-de-Mesquita HB, Ros MM, Kampman E, Egevad L, Overvad K, Tjonneland A, Roswall N, Clavel-Chapelon F, Boutron-Ruault MC, Touillaud M, Kaaks R, Chang-Claude J, Boeing H, Weikert S, Trichopoulou A, Naska A, Benetou V, Palli D, Sieri S, Vineis P, Tumino R, Panico S, van Duijnhoven FJ, Peeters PH, van Gils CH, Lund E, Gram IT, Sanchez MJ, Jakszyn P, Larranaga N, Ardanaz E, Navarro C, Rodriguez L, Manjer J, Ehrnstrom R, Hallmans G, Ljungberg B, Key TJ, Allen NE, Khaw KT, Wareham N, Slimani N, Jenab M, Boffetta P, Kiemeney LA, Riboli E (2011) Variety in vegetable and fruit consumption and risk of bladder cancer in the European Prospective Investigation into Cancer and Nutrition. International journal of cancer 128 (12):2971-2979. doi:10.1002/ijc.25636

24. Cooper AJ, Sharp SJ, Lentjes MA, Luben RN, Khaw KT, Wareham NJ, Forouhi NG (2012) A prospective study of the association between quantity and variety of fruit and vegetable intake and incident type 2 diabetes. Diabetes care 35 (6):1293-1300. doi:10.2337/dc11-2388

25. Jansen MC, Bueno-de-Mesquita HB, Feskens EJ, Streppel MT, Kok FJ, Kromhout D (2004) Quantity and variety of fruit and vegetable consumption and cancer risk. Nutrition and cancer 48 (2):142-148. doi:10.1207/s15327914nc4802_3

26. Buchner FL, Bueno-de-Mesquita HB, Ros MM, Overvad K, Dahm CC, Hansen L, Tjonneland A, Clavel-Chapelon F, Boutron-Ruault MC, Touillaud M, Kaaks R, Rohrmann S, Boeing H, Nothlings U, Trichopoulou A, Zylis D, Dilis V, Palli D, Sieri S, Vineis P, Tumino R, Panico S, Peeters PH, van Gils CH, Lund E, Gram IT, Braaten T, Sanchez MJ, Agudo A, Larranaga N, Ardanaz E, Navarro C,

Arguelles MV, Manjer J, Wirfalt E, Hallmans G, Rasmuson T, Key TJ, Khaw KT, Wareham N, Slimani N, Vergnaud AC, Xun WW, Kiemeney LA, Riboli E (2010) Variety in fruit and vegetable consumption and the risk of lung cancer in the European prospective investigation into cancer and nutrition. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology 19 (9):2278-2286. doi:10.1158/1055-9965.epi-10-0489

27. Kosti RI, Panagiotakos DB (2006) The epidemic of obesity in children and adolescents in the world. Central European journal of public health 14 (4):151-159

28. De Ferranti SD, Osganian SK (2007) Epidemiology of paediatric metabolic syndrome and type 2 diabetes mellitus. Diabetes & vascular disease research 4 (4):285-296. doi:10.3132/dvdr.2007.055

29. Lock K, Pomerleau J, Causer L, Altmann DR, McKee M (2005) The global burden of disease attributable to low consumption of fruit and vegetables: implications for the global strategy on diet. Bulletin of the World Health Organization 83 (2):100-108. doi:/S0042-96862005000200010

30. Boeing H, Bechthold A, Bub A, Ellinger S, Haller D, Kroke A, Leschik-Bonnet E, Muller MJ, Oberritter H, Schulze M, Stehle P, Watzl B (2012) Critical review: vegetables and fruit in the prevention of chronic diseases. European journal of nutrition 51 (6):637-663. doi:10.1007/s00394-012-0380-y

31. Lynch C, Kristjansdottir AG, Te Velde SJ, Lien N, Roos E, Thorsdottir I, Krawinkel M, de Almeida MD, Papadaki A, Hlastan Ribic C, Petrova S, Ehrenblad B, Halldorsson TI, Poortvliet E, Yngve A (2014) Fruit and vegetable consumption in a sample of 11-year-old children in ten European countries--the PRO GREENS cross-sectional survey. Public health nutrition 17 (11):2436-2444. doi:10.1017/s1368980014001347

32. Al Ani MF, Al Subhi LK, Bose S (2016) Consumption of fruits and vegetables among adolescents: a multi-national comparison of eleven countries in the Eastern Mediterranean Region. The British journal of nutrition 115 (6):1092-1099. doi:10.1017/s0007114515005371

33. Kimmons J, Gillespie C, Seymour J, Serdula M, Blanck HM (2009) Fruit and vegetable intake among adolescents and adults in the United States: percentage meeting individualized recommendations. Medscape journal of medicine 11 (1):26

34. Guenther PM, Dodd KW, Reedy J, Krebs-Smith SM (2006) Most Americans eat much less than recommended amounts of fruits and vegetables. Journal of the American Dietetic Association 106 (9):1371-1379. doi:10.1016/j.jada.2006.06.002

35. Agostinis-Sobrinho C, Santos R, Moreira C, Abreu S, Lopes L, Oliveira-Santos J, Rosario R, Povoas S, Mota J (2016) Association between serum adiponectin levels and muscular fitness in Portuguese adolescents: LabMed Physical Activity Study. Nutrition, metabolism, and cardiovascular diseases : NMCD. doi:10.1016/j.numecd.2016.02.011

36. Oliveira-Santos J, Santos R, Moreira C, Abreu S, Lopes L, Agostinis C, Mota J (2016) Ability of Measures of Adiposity in Identifying Adverse Levels of Inflammatory and Metabolic Markers in Adolescents. Childhood obesity (Print) 12 (2):135-143. doi:10.1089/chi.2015.0124

37. Trost SG, Loprinzi PD, Moore R, Pfeiffer KA (2011) Comparison of accelerometer cut points for predicting activity intensity in youth. Medicine and science in sports and exercise 43 (7):1360-1368. doi:10.1249/MSS.0b013e318206476e

38. Lopes C, Aro A, Azevedo A, Ramos E, Barros H (2007) Intake and adipose tissue composition of fatty acids and risk of myocardial infarction in a male Portuguese community sample. Journal of the American Dietetic Association 107 (2):276-286. doi:10.1016/j.jada.2006.11.008

39. Silva D, Rego C, Guerra A (2004) Characterization of food habits and comparative study between two methods of food assessment in adolescents. Rev Aliment Humana 10:33–40

40. Martins I, Porto A, Luísa O (2006) Table Composition of Foods. National Institute of Health Dr Ricardo Jorge, Lisbon, Portugal

41. Ferreira F, Graça M (1985) Composition Table of Portuguese Food. 2nd edn. National Institute of Health Dr Ricardo Jorge, Lisbon, Portugal

42. Goldberg GR, Black AE, Jebb SA, Cole TJ, Murgatroyd PR, Coward WA, Prentice AM (1991) Critical evaluation of energy intake data using fundamental principles of energy physiology: 1. Derivation of cut-off limits to identify under-recording. European journal of clinical nutrition 45 (12):569-581

43. Black AE (2000) Critical evaluation of energy intake using the Goldberg cut-off for energy intake:basal metabolic rate. A practical guide to its calculation, use and limitations. International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity 24 (9):1119-1130

44. Schofield WN (1985) Predicting basal metabolic rate, new standards and review of previous work. Human nutrition Clinical nutrition 39 Suppl 1:5-41

45. Trost SG, Ward DS, Moorehead SM, Watson PD, Riner W, Burke JR (1998) Validity of the computer science and applications (CSA) activity monitor in children. Medicine and science in sports and exercise 30 (4):629-633

46. Thompson FE, Willis GB, Thompson OM, Yaroch AL (2011) The meaning of 'fruits' and 'vegetables'. Public health nutrition 14 (7):1222-1228. doi:10.1017/s136898001000368x

47. Roark RA, Niederhauser VP (2013) Fruit and vegetable intake: issues with definition and measurement. Public health nutrition 16 (1):2-7. doi:10.1017/s1368980012000985

48. Cole TJ, Lobstein T (2012) Extended international (IOTF) body mass index cut-offs for thinness, overweight and obesity. Pediatric obesity 7 (4):284-294. doi:10.1111/j.2047-6310.2012.00064.x

49. Tanner JM, Whitehouse RH (1976) Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. Archives of disease in childhood 51 (3):170-179

50. Currie C, Molcho M, Boyce W, Holstein B, Torsheim T, Richter M (2008) Researching health inequalities in adolescents: the development of the Health Behaviour in School-Aged Children (HBSC) family affluence scale. Social science & medicine (1982) 66 (6):1429-1436. doi:10.1016/j.socscimed.2007.11.024

51. World Health Organization (1998) Guidelines for Controlling and Monitoring the Tobacco Epidemic. World Health Organization, Geneva

52. Slavin JL, Lloyd B (2012) Health benefits of fruits and vegetables. Advances in nutrition (Bethesda, Md) 3 (4):506-516. doi:10.3945/an.112.002154

53. Krebs-Smith SM, Kantor LS (2001) Choose a variety of fruits and vegetables daily: understanding the complexities. The Journal of nutrition 131 (2s-1):487s-501s

54. Padayatty SJ, Levine M (2008) Fruit and vegetables: think variety, go ahead, eat! The American journal of clinical nutrition 87 (1):5-7

55. Liu RH (2004) Potential synergy of phytochemicals in cancer prevention: mechanism of action. The Journal of nutrition 134 (12 Suppl):3479s-3485s

56. Kaulmann A, Bohn T (2014) Carotenoids, inflammation, and oxidative stress--implications of cellular signaling pathways and relation to chronic disease prevention. Nutrition research (New York, NY) 34 (11):907-929. doi:10.1016/j.nutres.2014.07.010

57. Rosa FT, Zulet MA, Marchini JS, Martinez JA (2012) Bioactive compounds with effects on inflammation markers in humans. International journal of food sciences and nutrition 63 (6):749-765. doi:10.3109/09637486.2011.649250

58. Liu RH (2003) Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. The American journal of clinical nutrition 78 (3 Suppl):517s-520s

59. Rangel-Huerta OD, Pastor-Villaescusa B, Aguilera CM, Gil A (2015) A Systematic Review of the Efficacy of Bioactive Compounds in Cardiovascular Disease: Phenolic Compounds. Nutrients 7 (7):5177-5216. doi:10.3390/nu7075177

60. Soeki T, Sata M (2016) Inflammatory Biomarkers and Atherosclerosis. International heart journal 57 (2):134-139. doi:10.1536/ihj.15-346

61. Chun OK, Chung SJ, Claycombe KJ, Song WO (2008) Serum C-reactive protein concentrations are inversely associated with dietary flavonoid intake in U.S. adults. The Journal of nutrition 138 (4):753-760

62. Julia C, Galan P, Touvier M, Meunier N, Papet I, Sapin V, Cano N, Faure P, Hercberg S, Kesse-Guyot E (2014) Antioxidant status and the risk of elevated C-reactive protein 12 years later. Annals of nutrition & metabolism 65 (4):289-298. doi:10.1159/000363194

63. Esmaillzadeh A, Kimiagar M, Mehrabi Y, Azadbakht L, Hu FB, Willett WC (2006) Fruit and vegetable intakes, C-reactive protein, and the metabolic syndrome. The American journal of clinical nutrition 84 (6):1489-1497

64. Holt EM, Steffen LM, Moran A, Basu S, Steinberger J, Ross JA, Hong CP, Sinaiko AR (2009) Fruit and vegetable consumption and its relation to markers of inflammation and oxidative stress in adolescents. Journal of the American Dietetic Association 109 (3):414-421. doi:10.1016/j.jada.2008.11.036

65. Root MM, McGinn MC, Nieman DC, Henson DA, Heinz SA, Shanely RA, Knab AM, Jin F (2012) Combined fruit and vegetable intake is correlated with improved inflammatory and oxidant status from a cross-sectional study in a community setting. Nutrients 4 (1):29-41. doi:10.3390/nu4010029

66. Wannamethee SG, Lowe GD, Rumley A, Bruckdorfer KR, Whincup PH (2006) Associations of vitamin C status, fruit and vegetable intakes, and markers of inflammation and hemostasis. The American journal of clinical nutrition 83 (3):567-574; quiz 726-567

67. Salas-Salvado J, Garcia-Arellano A, Estruch R, Marquez-Sandoval F, Corella D, Fiol M, Gomez-Gracia E, Vinoles E, Aros F, Herrera C, Lahoz C, Lapetra J, Perona JS, Munoz-Aguado D, Martinez-Gonzalez MA, Ros E (2008) Components of the Mediterranean-type food pattern and serum inflammatory markers among patients at high risk for cardiovascular disease. European journal of clinical nutrition 62 (5):651-659. doi:10.1038/sj.ejcn.1602762

68. Bhupathiraju SN, Tucker KL (2011) Greater variety in fruit and vegetable intake is associated with lower inflammation in Puerto Rican adults. The American journal of clinical nutrition 93 (1):37-46. doi:10.3945/ajcn.2010.29913

69. Dominguez Coello S, Cabrera de Leon A, Rodriguez Perez MC, Borges Alamo C, Carrillo Fernandez L, Almeida Gonzalez D, Garcia Yanes J, Gonzalez Hernandez A, Brito Diaz B, Aguirre-Jaime A (2010) Association between glycemic index, glycemic load, and fructose with insulin resistance: the CDC of the Canary Islands study. European journal of nutrition 49 (8):505-512. doi:10.1007/s00394-010-0110-2

70. Aeberli I, Hochuli M, Gerber PA, Sze L, Murer SB, Tappy L, Spinas GA, Berneis K (2013) Moderate Amounts of Fructose Consumption Impair Insulin Sensitivity in Healthy Young Men A randomized controlled trial. Diabetes care 36 (1):150-156

71. Aeberli I, Gerber PA, Hochuli M, Kohler S, Haile SR, Gouni-Berthold I, Berthold HK, Spinas GA, Berneis K (2011) Low to moderate sugar-sweetened beverage consumption impairs glucose and lipid metabolism and promotes inflammation in healthy young men: a randomized controlled trial. The American journal of clinical nutrition 94 (2):479-485

72. Stanhope KL (2016) Sugar consumption, metabolic disease and obesity: The state of the controversy. Crit Rev Clin Lab Sci 53 (1):52-67. doi:10.3109/10408363.2015.1084990

73. Martinez-Gomez D, Eisenmann JC, Warnberg J, Gomez-Martinez S, Veses A, Veiga OL, Marcos A (2010) Associations of physical activity, cardiorespiratory fitness and fatness with low-grade inflammation in adolescents: the AFINOS Study. International journal of obesity (2005) 34 (10):1501-1507. doi:10.1038/ijo.2010.114

74. Perez CM, Ortiz AP, Fuentes-Mattei E, Velazquez-Torres G, Santiago D, Giovannetti K, Bernabe R, Lee MH, Yeung SC (2014) High prevalence of cardiometabolic risk factors in Hispanic adolescents: correlations with adipocytokines and markers of inflammation. Journal of immigrant and minority health / Center for Minority Public Health 16 (5):865-873. doi:10.1007/s10903-013-9866-9

75. Raynor HA, Osterholt KM (2012) Greater variety of fruit served in a four-course snack increases fruit consumption. Appetite 59 (3):662-667. doi:10.1016/j.appet.2012.08.003

76. Raynor HA, Epstein LH (2001) Dietary variety, energy regulation, and obesity. Psychological bulletin 127 (3):325-341

77. Tremblay MS, Colley RC, Saunders TJ, Healy GN, Owen N (2010) Physiological and health implications of a sedentary lifestyle. Applied physiology, nutrition, and metabolism = Physiologie appliquee, nutrition et metabolisme 35 (6):725-740. doi:10.1139/h10-079

78. Nimmo MA, Leggate M, Viana JL, King JA (2013) The effect of physical activity on mediators of inflammation. Diabetes, obesity & metabolism 15 Suppl 3:51-60. doi:10.1111/dom.12156

Table 1: Participants' characteristics of Portuguese adolescents from the LabMed Physical Activity Study

			All [a] (n=412)	Girls [a] (n=216)	Boys [a] (n=196)	P value [b]
Age (years)			14.9 (12.6 – 15.7)	14.9 (12.6 – 15.6)	15.0 (12.7 – 15.8)	0.143
Pubertal stage (Tanner A)	2		7.2%	33.3%	11.7%	**0.001**
	3		33.0%	54.6%	36.2%	
	4		47.1%	12.0%	38.8%	
	5		12.6%	2.8%	13.3%	
Pubertal stage (Tanner B)	2		7.0%	22.7%	11.7%	**<0.001**
	3		20.6%	46.8%	18.4%	
	4		50.2%	27.8%	54.1%	
	5		22.1%	3.2%	15.8%	
Body mass index	underweight		3.6%	3.7%	3.6%	0.574
	normal weight		66.7%	65.3%	68.4%	
	overweight		22.1%	21.8%	22.4%	
	obese		7.5%	9.3%	5.6%	
Socioeconomic status			6.0 (5.0 – 8.0)	6.0 (6.0 – 8.0)	6.0 (5.0 – 8.0)	0.416
Sedentary Time (minutes.day^{-1}) [c]			667.4 (619.4 – 725.3)	678.4 (632.8 – 734.1)	645.9 (607.5 – 713.2)	**0.003**
Moderate-to-vigorous physical activity (minutes.day^{-1}) [c]			51.0 (39.1 – 65.3)	45.5 (35.1 – 59.5)	56.7 (43.0 – 71.5)	**<0.001**
Smoking habits [d]	Current smokers		1.0%	0.9%	1.0%	**0.037**
	Occasional smokers		1.7%	1.4%	2.0%	
	Former smokers		7.8%	4.6%	11.2%	
	Non-smokers		89.6%	93.1%	85.7%	
Energy intake (kcal.day^{-1})			2 063 (1 598 – 2 593)	2 016 (1 559 – 2 404)	2 123 (1 648 – 2 730)	**0.005**
Vegetable intake	Quantity (g/day)		107.4 (47.0 – 180.3)	95.8 (40.8 – 177.4)	120.7 (55.3 – 183.5)	0.264
	Variety (categories/month)		10.0 (5.0 – 13.0)	9.0 (5.0 – 12.0)	10.0 (6.0 – 14.0)	**0.004**
	Variety tertiles	1st: ≤6	33.3%	38.4%	27.6%	**0.012**
		2nd: 7 - 12	36.9%	37.5%	36.2%	
		3rd: ≥13	29.9%	24.1%	36.2%	
Fruit intake	Quantity (g/day)		192.7 (115.0 – 307.8)	204.4 (113.4 – 320.7)	188.9 (119.3 – 290.4)	0.735
	Variety (categories/month)		10.0 (7.0 – 11.0)	10.0 (7.0 – 11.0)	10.0 (8.0 – 12.0)	**0.016**
	Variety tertiles	1st: ≤9	43.7%	47.7%	39.3%	**0.009**
		2nd: 10 - 11	34.7%	36.6%	32.7%	
		3rd: =12	21.6%	15.7%	28.1%	
CRP (mg/L)			0.20 (0.11 – 0.77)	0.11 (0.11 – 0.49)	0.34 (0.11 – 1.26)	**<0.001**
IL-6 (ng/L)			1.90 (1.90 – 3.40)	1.90 (1.90 – 3.40)	1.90 (1.90 – 3.35)	0.561
C3 (mg/dL)			116.0 (107.0 – 126.5)	119.0 (107.0 – 127.0)	115.0 (106.5 – 126.0)	0.888
C4 (mg/dL)			20.0 (16.0 – 24.0)	20.0 (16.0 – 25.0)	20.0 (17.0 – 24.0)	0.561
Overall inflammatory biomarker score [e]			1.78 (1.00 – 3.00)	1.79 (1.00 – 3.00)	1.77 (1.00 – 3.00)	0.884

Abbreviations: CRP – C-reactive protein, IL-6 – Interleukin-6, C3 - Complement component 3, C4 - Complement component 4.
[a] The data shown column percentage for categorical variables and median (25th -75th percentiles) for continuous variables.
[b] P value was calculated based on Qui-squared test for categorical variables and Mann–Whitney U test for continuous variables.
[c] It was considered n=329 (55.9% girls), because there is some missing values in Sedentary time and Moderate-to-vigorous physical activity variables.
[d] Qui-squared test performed with "Current smokers" and "Occasional smokers" together to improve power of test.
[e] Overall inflammatory biomarker score were designed summing the inflammatory biomarkers (CRP, IL-6, C3 and C4) categories, wherein for each category was assigned one point if the biomarker was above the median adjusted by age and sex or zero if below the median.

Table 2: Differences between inflammatory biomarkers categories across fruit and vegetable variety tertiles

			Vegetable variety (categories/month)				**Fruit variety** (categories/month)			
			1st tertile: ≤6	2nd tertile: 7 - 12	3rd tertile: ≥13	p[a]	1st tertile: ≤9	2nd tertile: 10 - 11	3rd tertile: =12	p[a]
CRP (mg/L)	Lower		**50.7%**	**43.7%**	**69.9%**	**<0.001**	47.6%	55.6%	61.0%	0.123
	Higher		**49.3%**	**56.3%**	**30.1%**		52.4%	44.4%	39.0%	
IL-6 (ng/L)	Lower		**65.4%**	**56.3%**	**71.5%**	**0.003**	69.9%	59.9%	62.2%	0.161
	Higher		**34.6%**	**43.7%**	**28.5%**		30.1%	40.1%	37.8%	
C3 (mg/dL)	Lower		**55.9%**	**41.7%**	**58.5%**	**0.010**	52.4%	52.4%	48.8%	0.840
	Higher		**44.1%**	**58.3%**	**41.5%**		47.6%	47.6%	51.2%	
C4 (mg/dL)	Lower		54.4%	45.7%	58.5%	0.091	56.6%	49.2%	53.7%	0.398
	Higher		45.6%	54.3%	41.5%		43.4%	50.8%	46.3%	
Overall inflammatory biomarker score [b]	0-1	Biomarkers above median	**44.9%**	**35.8%**	**56.1%**	**0.003**	46.2%	43.3%	46.3%	0.840
	2-4		**55.1%**	**64.2%**	**43.9%**		53.8%	56.7%	53.7%	

Abbreviations: CRP – C-reactive protein, IL-6 – Interleukin-6, C3 - Complement component 3, C4 - Complement component 4.

[a] The p value was calculated based on Qui-square test.

[b] Overall inflammatory biomarker score were designed summing the inflammatory biomarkers (CRP, IL-6, C3 and C4) categories, wherein for each category was assigned one point if the biomarker was above the median adjusted by age and sex or zero if below the median.

Table 3: Association between vegetable and fruit variety tertiles and inflammatory biomarkers categories

	Vegetable variety: OR (95% CI)				**Fruit variety:** OR (95% CI)			
	1st tertile ≤ 6	2nd tertile 7 to 12	3rd tertile ≥ 13	P_{trend} [a]	1st tertile ≤ 9	2nd tertile 10 to 12	3rd tertile ≥ 13	P_{trend} [a]
CRP models								
Crude	1.00	1.36 (0.86-2.17)	**0.45 (0.27-0.75)**	**0.006**	1.00	0.87 (0.56-1.35)	0.74 (0.45-1.24)	0.282
Adjusted 1[b]	1.00	1.33 (0.84-2.13)	**0.42 (0.25-0.71)**	**0.004**	1.00	0.87 (0.56-1.35)	0.71 (0.42-1.20)	0.239
Adjusted 2[b,c]	1.00	1.14 (0.65-1.99)	**0.32 (0.17-0.61)**	**0.002**	1.00	0.97 (0.57-1.64)	0.62 (0.33-1.17)	0.315
Adjusted 3[b,c]	1.00	1.23 (0.63-2.02)	**0.31 (0.15-0.64)**	**0.004**	1.00	1.12 (0.65-1.93)	0.74 (0.39-1.42)	0.729
IL-6 models								
Crude	1.00	1.51 (0.94-2.43)	0.76 (0.45-1.29)	0.452	1.00	1.01 (0.64-1.59)	1.12 (0.66-1.90)	0.773
Adjusted 1[b]	1.00	1.53 (0.95-2.47)	0.79 (0.46-1.34)	0.538	1.00	1.01 (0.64-1.60)	1.17 (0.69-1.99)	0.807
Adjusted 2[b,c]	1.00	1.57 (0.90-2.74)	0.73 (0.38-1.38)	0.501	1.00	1.04 (0.61-1.79)	1.74 (0.92-3.30)	0.253
Adjusted 3[b,c]	1.00	1.62 (0.91-2.90)	0.76 (0.38-1.54)	0.623	1.00	1.10 (0.63-1.93)	**2.16 (1.11-4.20)**	0.066
C3 models								
Crude	1.00	**1.77 (1.12-2.82)**	0.91 (0.56-1.49)	0.910	1.00	1.04 (0.67-1.62)	1.14 (0.69-1.90)	0.662
Adjusted 1[b]	1.00	**1.77 (1.11-2.83)**	0.92 (0.56-1.51)	0.947	1.00	1.04 (0.67-1.62)	1.16 (0.70-1.94)	0.635
Adjusted 2[b,c]	1.00	1.78 (0.99-3.18)	0.62 (0.33-1.18)	0.286	1.00	1.08 (0.63-1.86)	1.36 (0.71-2.60)	0.451
Adjusted 3[b,c]	1.00	1.84 (1.00-3.37)	0.66 (0.32-1.36)	0.435	1.00	1.15 (0.66-2.00)	1.48 (0.76-2.88)	0.320
C4 models								
Crude	1.00	1.42 (0.89-2.25)	0.86 (0.52-1.40)	0.668	1.00	**1.64 (1.06-2.56)**	1.17 (0.70-1.95)	0.111
Adjusted 1[b]	1.00	1.41 (0.89-2.25)	0.85 (0.52-1.40)	0.660	1.00	**1.64 (1.06-2.56)**	1.17 (0.69-1.96)	0.110
Adjusted 2[b,c]	1.00	1.32 (0.77-2.25)	0.76 (0.42-1.36)	0.452	1.00	**1.71 (1.02-2.85)**	1.46 (0.79-2.68)	0.057
Adjusted 3[b,c]	1.00	1.50 (0.85-2.64)	0.94 (0.48-1.82)	0.967	1.00	**1.96 (1.15-3.33)**	1.72 (0.91-3.23)	**0.016**
Overall inflammatory biomarker score models [d]								
Crude	1.00	1.50 (0.94-2.41)	0.65 (0.40-1.06)	0.136	1.00	1.09 (0.70-1.70)	0.98 (0.59-1.63)	0.869
Adjusted 1[b]	1.00	1.50 (0.93-2.41)	0.65 (0.39-1.06)	0.140	1.00	1.09 (0.70-1.70)	0.99 (0.59-1.65)	0.852
Adjusted 2[b,c]	1.00	1.52 (0.86-2.70)	**0.47 (0.25-0.87)**	**0.044**	1.00	1.10 (0.65-1.89)	1.22 (0.65-2.32)	0.545
Adjusted 3[b,c]	1.00	1.76 (0.96-3.23)	0.61 (0.30-1.22)	0.272	1.00	1.24 (0.72-2.15)	1.44 (0.74-2.78)	0.268

Abbreviations: OR – odds ratio, CI – confidence interval, CRP – C-reactive protein, IL-6 – Interleukin-6, C3 – Complement component 3, C4 – Complement component 4.

[a] Tests of linear trend were conducted for each model by assigning the median values of vegetable or fruit variety for each tertile to all participants in the tertile as an ordinal variable. The p-value of this variable was interpreted as the p-value for trend.

[b] Models adjustment were: (1) for sex; (2) for sex, age, pubertal stage (tanner A and B), body mass index, energy intake, socio-economic status, sedentary time, moderate-to-vigorous physical activity, and smoking habits; (3) for the same variables of model 2 plus fruit quantity for fruit variety

models or vegetable quantity for vegetable variety models.

[c] These models were performed with n=329 (55.9% girls) because there is some missing values in Sedentary time and Moderate-to-vigorous physical activity variables.

[d] Overall inflammatory biomarker score were designed summing the inflammatory biomarkers (CRP, IL-6, C3 and C4) categories, wherein for each category was assigned one point if the biomarker was above the median adjusted by age and sex or zero if below the median.

7.4. PAPER IV

Dietary inflammatory index and inflammatory biomarkers in adolescents from LabMed physical activity study.

[Submitted for publication]

Dietary Inflammatory Index and Inflammatory Biomarkers in Adolescents from LabMed Physical Activity Study

RUNNING TITLE:

Dietary Inflammatory Index in Adolescents

AUTHORS:

Almeida-de-Souza, Juliana. School of Health, Polytechnic Institute of Bragança, Portugal. Faculty of Nutrition and Food Sciences, University of Porto, Porto, Portugal.

Santos, Rute. Research Centre in Physical Activity, Health and Leisure, Faculty of Sport, University of Porto, Portugal. Early Start Research Institute, University of Wollongong, Australia.

Barros, Renata. Faculty of Nutrition and Food Sciences, University of Porto, Porto, Portugal.

Abreu, Sandra. Research Centre in Physical Activity, Health and Leisure, Faculty of Sport, University of Porto, Portugal.

Moreira, Carla. Research Centre in Physical Activity, Health and Leisure, Faculty of Sport, University of Porto, Portugal.

Lopes, Luís. Research Centre in Physical Activity, Health and Leisure, Faculty of Sport, University of Porto, Portugal.

Moreira, Pedro. Faculty of Nutrition and Food Sciences, University of Porto, Porto, Portugal. Institute of Public Health, University of Porto, Porto, Portugal

CORRESPONDENCE TO:

Juliana Almeida-de-Souza. School of Health, Polytechnic Institute of Bragança. Av. D. Afonso V – 5300-121 Bragança, Portugal. Telephone number: 273 330 950; Fax number: 273 327 915; e-mail: julianaalmeida@ipb.pt

DISCLOSURE STATEMENTS:

The authors disclose that LabMed Physical Activity Study was supported by *Fundação para a Ciência e a Tecnologia* (FCT) grants: BPD/102381/2014. The Research Centre on Physical Activity Health and Leisure (CIAFEL) is supported by UID/DTP/00617/2013 (FCT). This paper is part of PhD thesis in Faculty of Nutrition and Food Science, University of Porto, Porto, Portugal

CONFLICT OF INTEREST STATEMENT:

The authors declare there are no conflicts of interest.

ABSTRACT

BACKGROUND/OBJECTIVES: The Dietary Inflammatory Index (DII) is a tool to measure the diet's inflammatory potential and has been used with adults to predict low-grade inflammation. The present study aims to assess whether this dietary score predicts low-grade inflammation in adolescents.

SUBJECTS/METHODS: The sample comprises 412 adolescents (52.4% girls), aged 12-18 years, from LabMed Physical Activity Study. DII score was calculated based on a food-frequency questionnaire and categorized into tertiles. We collected blood samples to determine the follow inflammatory biomarkers: C-reactive protein (CRP), interleukin-6 (IL-6), complement component 3 (C3), and 4 (C4). In addition we calculated an overall inflammatory biomarker score. Odds ratios (OR) and 95% confidence intervals (95%CI) were computed from binary logistic regression models.

RESULTS: DII score, comparing first with third tertile, was positively associated with IL-6 in all models: crude (OR=1.88, 95%CI:1.09-3.24, p_{trend}=0.011), adjusted for sex (OR=1.84, 95%CI:1.06-3.18, p_{trend}=0.015) and fully adjusted (for biological and lifestyle variables) (OR=3.38, 95%CI:1.24-9.20, p_{trend}=0.023). Also, DII score was positively associated with C4, when fully adjusted (0R=3.12, 95%CI:1.21-8.10, p_{trend}=0.016). No significant associations were observed between DII score and CRP or C3. However, DII score was positively associated with the overall inflammatory biomarker score, when fully adjusted (OR=5.61, 95%CI:2.00-15.78, p_{trend}=0.002).

CONCLUSIONS: DII score can be useful to assess the diet's inflammatory potential and its association with low-grade inflammation in adolescents.

INTRODUCTION

Low-grade inflammation correlates with a set of chronic conditions[1, 2] such as obesity,[3] diabetes,[3, 4] cardiovascular diseases,[5, 6] and cancer.[7, 8] This association has also been found in youth for obesity,[9-11] central obesity,[12, 13] metabolic syndrome,[14, 15] atherosclerosis,[16] and several other cardiovascular risk factors.[10, 13] In addition, systemic inflammation in childhood and adolescence is known to continue into adulthood.[17]

Inflammatory status is heavily reliant on the measurement of the inflammatory biomarkers, such as acute phase proteins and cytokines,[1] and a number of modifying factors, including diet, have been shown to influence the inflammatory status in the life cycle.[1, 2]

Studies about nutrition intake and low-grade inflammation in adolescents are scarce. However, evidence suggests that the consumption of fat and antioxidant vitamins (vitamins E, C, and beta-carotene) are determinants of low-grade inflammation during adolescence.[18] While antioxidant vitamins[19, 20] and polyunsaturated fatty acids[19] seem to have anti-inflammatory properties, total fat[20] and saturated fat acids[19, 20] seems to be pro-inflammatory. Moreover, a Western dietary pattern is considered to be pro-inflammatory diet.[19]

A dietary pattern approach has been considered advantageous because it considers synergistic or antagonistic interactions among nutrients and other food components.[21] The Dietary Inflammatory Index (DII)[22, 23] is a score that attempts to combine the inflammatory property of each nutrient or food component of the diet.

The DII was designed by Cavicchia[22] and updated by Shivappa,[23] and it is a tool to measure the diet's inflammatory power, scoring individuals' diets from maximal anti-inflammatory to maximal pro-inflammatory. This index was initially (in the first version) correlated to C-reactive protein (CRP) in apparently healthy adults[22] and seniors[24] (in an adapted first version) and also to other inflammatory biomarkers such as interleukin-6 (IL-6) and a combined inflammatory biomarker score in adults and seniors.[25] For the updated version, several studies have found associations between DII score and CRP, in adults and seniors,[26] and IL-6, tumour necrosis factor-alpha (TNF-α) and another score of combined inflammatory biomarker in postmenopausal women.[27] The DII score has been used in several studies to predict mortality,[28, 29] survival[30], and diseases, especially cancer,[30, 31] but also obesity,[32] cardiovascular disease,[33] and metabolic syndrome.[32, 34]

Considering that the published studies using the DII score were predominantly conducted with adults or seniors, and no study was found to focus on adolescents, this paper aims to assess the association between DII score and inflammatory biomarkers in adolescents.

SUBJECTS AND METHODS

Study design and sampling

We used baseline data, collected in 2011, from the Longitudinal Analysis of Biomarkers and Environmental Determinants of Physical Activity Study (LabMed Physical Activity Study). The LabMed Physical Activity Study is a school-based

prospective cohort study carried out in five schools from the north of Portugal, which aimed to evaluate the independent and combined associations of dietary intake and fitness levels on blood pressure levels of adolescents.

The LabMed Physical Activity study was conducted in accordance with the World Medical Association's Helsinki Declaration for Human Studies. The Portuguese Data Protection Authority (#1112434/2011), the Portuguese Ministry of Science and Education (0246200001/2011) and Faculty of Sport, University of Porto, approved the study. All participants were informed of the study's goals, and written informed consent was obtained from participating adolescents and their parents or guardians.

Considering combined healthy diet/physical activity pattern prevalence of 14%,[35] we calculated a minimum sample size of 1086 subjects for have a power of 80%, to detect 15% difference between exposed and unexposed, at 5% significance, considering an expected dropout rate of about 20%.

From an initial total sample of 1229 apparently healthy adolescents (12–18 years), 534 accepted to undergo blood collection. Of these, 412 adolescents had completed and accurate data on dietary intake. We found no differences in most variables between those who accepted or not to undergo blood sampling. However, boys, olders and daily smokers reject more undergoing blood sampling.

130 Inflammatory biomarkers assessment and overall inflammatory biomarker
131 score

132 Blood samples were collected from the antecubital vein, at least ten hours of
133 fasting conditions, refrigerated (4º–8°C), and sent to laboratory for measure
134 inflammatory biomarkers: CRP by the latex-enhanced turbidimetric assay
135 (Siemens Advia 1600/1800, Erlangen, Germany); IL-6 by the chemiluminescence
136 immunoassay (Immulite 2000, Diagnostic Products Corporation, Los Angeles,
137 CA); complement component 3 (C3) and 4 (C4) by the Immunoturbidimetric
138 assay (Siemens Advia 1600/1800, Erlangen, German).

139 Inflammatory biomarkers were dichotomized, based on sex- and age-adjusted
140 median values, because they have a very skewed distribution, and no cut-offs
141 values is established for adolescents. Categories considered were higher or
142 lower inflammatory state. Medians of each category (higher/lower) were
143 defined as follows: 0.11/0.92 mg/L for CRP, 1.90/4.20 ng/L for IL-6,
144 107.00/127.00 mg/dL for C3, and 17.00/25.55 mg/dL for C4.

145 We created an overall inflammatory biomarker score considering each
146 biomarker categories, assigning one point to those who were above the sex- age-
147 adjusted median or zero for those who were below, and summing all points
148 assigned. The overall inflammatory biomarker score varies from zero to four
149 inflammatory biomarkers above the median. We also create two categories: 0–1
150 (49.9%) or 2–4 (50.1%) biomarkers above the median.

151

Physical activity and sedentary time assessment

The physical activity and sedentary time were assessed with accelerometers GT1M (ActiGraph, Pensacola, Florida, USA). Participants were instructed to use the accelerometer attached on the right side of hip, with the notch faced upwards, over five consecutive days (three weekdays, two weekend days) during waking hours and remove it during water-based activities. The epoch length was set to 2 seconds to allow a more detailed estimate of physical activity intensity.

Accelerometer data were analysed by an automated data reduction program (ActivLive 6.12, ActiGraph, Pensacola, Florida, USA). Periods with 60 minutes of consecutive zeros were detected and flagged non-wear time. Participants had to have at least 8 hours of data to count as a valid day and to have at least three valid days (two weekdays, one weekend day) to be included. This combination of hours and days were studied to achieve a reliability of 90%.[36]

After screening, the raw activity "counts" was processed for determination of time spent in the different physical activity intensities. Physical activity was expressed as the time spent in moderate-to-vigorous physical activity. The cut-points recommend by American College of Sports Medicine[37] were used and we identify moderate-to-vigorous physical activity and sedentary time was expressed in minutes.day^{-1}.

Dietary intake assessment

A self-administered semi-quantitative food-frequency questionnaire validated for Portuguese population,[38] and adapted to adolescents,[39] was used to measure

the dietary intake. The food-frequency questionnaire lists 91 options of food and beverage items or categories, and assesses the food habits in previous 12 months. For each option, there are nine response possibilities (from "never or less than once per month" to "six or more times per day"), standard portion sizes, and a seasonality choice. In the end, there is still space available for each respondent include any food not listed. Dietary intake estimation was made multiplying the portion size in grams by the multiple/fraction of daily frequency intake and by a seasonality variation factor for each option selected. The conversion, from food to energy and nutrients intake, was performed using The Food Processor Plus program version SQL (ESHA Research, Salem, OR, USA). This database was supplemented with the Portuguese food composition database.[40]

To determine the misreporting of dietary assessment, implausible energy intake was calculated using the Goldberg' method, adapted by Black.[41] First, the basal metabolic rate was calculated using Schofield equation, considering sex and age. Second, a ratio energy intake/basal metabolic rate was compared to the 95% confidence limits (cut-offs). The cut-offs were calculated using our sample specific values for: mean of physical activity level, number of days of dietary assessment, within-subject coefficient of variation in energy intake, between-subject variation in physical activity, and variation in basal metabolism rate. The physical activity level was calculated in using counts.minutes^{-1} and time of daily use from accelerometers, as Trost formula,[42] reaching a value of 1.23. A number of 21 days of diet assessment was considered, as Black recommendation for food-frequency questionnaire.[41] The within-subject coefficient of variation in energy intake was calculated considering mean and standard deviation of energy

intake in our sample. Between-subject variation in physical activity was calculated considering mean and standard deviation of physical activity level in our sample. A figure of 8.5% was used for the coefficient of variation of repeated basal metabolic rate measurements, as Black suggested.[41] The cut-offs calculated were 0.61–2.48; accordingly, a total of 150 adolescents with energy intake/basal metabolic rate below 0.61 and over 2.48 were considered as misreporting of dietary assessment and were excluded.

Energy intake was expressed in $kj.day^{-1}$ and $kcal.day^{-1}$.

Food-frequency questionnaire data was also used to calculate DII score.

Description of the Dietary Inflammatory Index score

The DII is a literature-based tool[23] that measures the diet' inflammatory properties by a score, and it is based on review about the role of food and dietary constituents on follow inflammatory biomarkers: CRP, TNF-α and Interleukin's 1β, 4, 6 and 10. The review pointed 45 food parameters and they were scored with +1, -1 or 0 according to their inflammatory effects: pro, anti or null, respectively. The number of articles and the type of study were also used to weight each one of 45 food parameters and calculate a "food parameter-specific overall inflammatory effect score", used as multiplying factors, to calculate a DII score. The final score, ranging from -8.87 to 7.98, is interpreted as strongly anti-inflammatory to strongly pro-inflammatory, respectively.

In this study, the DII score was calculated considering 31 food parameters. Eugenol, garlic, ginger, saffron, turmeric, flavan-3-ol, flavones, flavonols, flavonones, anthocyanidis, isoflavones, pepper, thyme/oregano, rosemary were

not included because no information was available for these components in the Food Processor nutritional database neither those herbs or spices were included in the food-frequency questionnaire.

Briefly describing the DII score calculation according to Shivappa,[23] first a mean and standard deviation were calculated for the 31 food parameters available (table 1), because no global data for adolescent is available. Second, a z-score of each food parameter and for each participant was calculated. Third, the individual z-score were converted to a centred percentile. Fourth, each centred percentile were multiplied by its respective food parameter-specific overall inflammatory effect score, published by Shivappa[23], and the food parameter-specific DII score is obtained. Finally, the 31 food parameter-specific DII score was summed and an individual DII score was obtained. All food parameters are correlated ($p<0.001$) with DII score; caffeine, alcohol and green/black tea are food parameters with weakest correlation; while magnesium, vitamin B6 and dietary fibre are food parameters with strongest correlations (table 1).

Our DII score values was ranging from -5.36 to 4.24, and it was categorized, based on tertiles values, in accordance with Shivappa,[26] considering Low (1st tertile: <-1.34), Medium (2nd tertile: -1.34 to 1.41) and High (3rd tertile: >1.41) pro-inflammatory dietary property.

Anthropometric assessment data

Height and weight were measured with a portable stadiometer (SECA 213, Hamburg, Germany) and a portable scale (TANITA Inner Scan BC532, Tokyo, Japan), respectively. Adolescents should be standing upright, lightly dressed and

no shoes. Body mass index was calculated from the weight (kg) to height squared (m^2) ratio and participants were classified as underweight, normal weight, overweight and obese.[43]

Pubertal stage

Pubertal stage, from 1 to 5, was self-assessed relatively the secondary sex characteristics, according to Tanner and Whitehouse criteria.[44]

Socio-economic status

Socio-economic status was self-assessed with Family Affluence Scale,[45] ranking from 0 to 9, considering lower scores as lower socio-economic status.

Smoking habits

Smoking habits was self-reported and participants were classified according World Health Organization criteria[46] as: non-smokers, former smokers, occasional smokers, and current smokers.

Statistical Analyses

Participants' characteristics are presented as percentages, medians and inter-quartiles range. Mann-Whitney U test, Qui-square test and Spearman's correlation were used to assess associations between variables.

To study the association between DII score tertiles and inflammatory biomarkers, fifteen binary logistic regression models were constructed. There were three models (crude, sex-adjusted and fully adjusted) for each inflammation biomarker and for the overall inflammatory biomarker score, as dependent variables, and DII score tertiles, as predictor. Fully adjusted model were adjusted for sex, age, pubertal stage (Tanner A and B), body mass index, energy intake, socio-economic status, sedentary time, moderate-to-vigorous physical activity and smoking habits. Multicollinearity was tested, and no multicollinearity between independent variables was observed. *Post hoc* power calculations were performed considering our minimal sample size (n=329), our minimal odds ratio (OR=3.12), a null hypothesis value of 0.5, and 5% significance, achieving a power of 0.99.

A 0.05 level of significance and 95%CI (confidence interval) were considered. Data was analysed using the statistical package SPSS®, version 21.0 (SPSS Inc., Chicago, IL, USA) and power were calculated using G*power, version 3.1 (Faul, Erdfelder, Lang, & Buchner, 2007).

RESULTS

Girls presented on average a higher DII score and lower CRP than boys (table 2).

The IL-6 was positively correlated with DII score and adolescents within the 3rd tertile of the DII score had higher prevalence of higher IL-6 than adolescents within the 1st or 2nd tertiles. No significant differences or correlations were observed for the other biomarkers or for the overall inflammatory biomarker score and DII score (table 3).

Table 4 shows that adolescents within the 3rd tertile of the DII score showed significantly higher odds of having higher IL-6 in all models: crude (OR=1.88, 95%CI:1.09-3.24, p_{trend}=0.011), sex-adjusted (OR=1.84, 95%CI:1.06-3.18, p_{trend}=0.015), and fully adjusted (OR=3.38, 95%CI:1.24-9.20, p_{trend}=0.023). Also, for fully adjusted models, adolescents within the 3rd tertile of the DII score showed significantly higher odds of having higher C4 (0R=3.12, 95%CI:1.21-8.10, p_{trend}=0.016), and overall inflammatory biomarker score (OR=5.61, 95%CI:2.00-15.78, p_{trend}=0.002).

DISCUSSION

To the best of our knowledge, this is the first study exploring associations between the DII score and inflammatory biomarkers in adolescents.

We showed that DII score predicted low-grade inflammation, specifically IL-6, C4 and the overall inflammatory biomarker score, in adolescents. In our study, the DII score was independently and positively correlated with IL-6, and adolescents whose diets showed low- or medium-inflammatory properties had a lower prevalence of higher IL-6. Also, in the fully adjusted regression model, when comparing DII score 1st tertile (low-inflammatory diet) with the 3rd tertile (high-inflammatory diet), the odds of having higher IL-6 was about three times higher. These findings seem to be important since IL-6 is considered a more sensitive indicator of cardiovascular disease than others like CRP.[47, 48]

In our study, DII score was not associated with CRP, contrary to what we were expecting considering the DII scores conception (a literature-based tool about

316 the role of diet on inflammatory biomarkers, including CRP)[22, 23] and
317 validation,[22, 26] but consistent with other studies in adults.[25, 27] However, some
318 studies have found this relationship, particularly those conducted with
319 apparently healthy adults,[22, 26, 34] or seniors.[24] In this regard, it is important to
320 notice that in these studies, the CRP mean levels were much higher than in our
321 sample. For example, in the SEASONS cohort,[26] CRP mean ranged from 2.2±5.1 to
322 2.2±5.7mg/L in women and from 2.3±4.4 to 2.4±4.6mg/L in men, whereas in our
323 study, the corresponding values were 0.83±2.24mg/L (girls) and 1.62±4.54mg/L
324 (boys). Moreover, only 7% of the participants in our study presented CRP levels
325 between 3 and 10mg/L, while in the SEASONS cohort, this prevalence reached
326 18%. Another important concept to be noted is the number of modifying factors
327 related to the inflammatory biomarkers such as age or body fatness.[1, 2] Again
328 comparing again our study to the SEASONS cohort,[26] our age range is 12–18
329 years, while the SEASONS cohort is 20–70 years; by contrast, our prevalence of
330 normal BMI is about 65% (girls) and 68% (boys), while the SEASONS cohort was
331 about 44% (women) and 30% (men). Thus, with all of these parameters
332 described, differences in the prevalence of CRP inadequacy and the presence of
333 modifying factors of inflammatory biomarkers between samples may help to
334 explain the differences in the association between DII score and CRP across the
335 studies.

336 We also found an association between the DII score with C4 and the overall
337 inflammatory biomarker score, in line with some authors[25, 27] who found a
338 relationship between DII score and a different inflammatory biomarker scores
339 only for the fully adjusted model. This means that adolescents with a high pro-
340 inflammatory diet have an odds ratio five times higher of having two to four

biomarkers above the median. However, these associations are true only for the fully adjusted model. Calder et al.[1] discuss how modifying factors can affect the concentration of inflammatory biomarkers, and we try to control the effect of most of the possible variables in the fully adjusted models, such as age and pubertal stage; body mass index as a measurement of body fatness; sedentary time and moderate-to-vigorous physical activity as measurements of physical (in)activity; sex, smoking habits and socio-economic status. These factors together must have a significant impact on inflammatory biomarkers, masking the association between DII score with C4 and with the overall inflammatory biomarker score in the crude models. However, when we control those variables, the association between DII score with C4 and the overall inflammatory biomarker score can be observed.

Additionally, we found an association between DII score and the overall inflammatory biomarker score, but not for CRP or C3, although the trend is significant for C3. Furthermore, the overall inflammatory biomarker score had showed a higher odds ratio (OR=5.61) than IL-6 (OR=3.38) or C3 (OR=3.12). In fact, the inflammatory biomarkers in general are considered non-specific pro-inflammatory response markers in healthy people, and the biomarkers' signatures that best represent low-grade inflammation are yet to be fully understood.[2] Our overall inflammatory biomarker score is a more complex and integrated assessment of low-grade inflammation, rather than just an inflammatory biomarker alone. This score takes into account the sums of the effects of all inflammatory biomarkers, that is, those that were shown to have a relationship with the DII (IL-6 and C4) and those that did not (CRP and C3), and it seems to represent better low-grade inflammation in this group of adolescents.

366 The strengths of this study include the novelty of its aim and the use of
367 objectively measured physical activity and sedentary time. We also included
368 sedentary time as a covariate in our models once it was considered a risk factor
369 for cardiovascular health independently of physical activity levels.[49] In addition,
370 we use only accurate food-frequency questionnaires, according to Goldberg'
371 method.[41] That method is useful to evaluate the mean population bias in
372 reporting energy intake and recommends the use of information about physical
373 activity, as we do. Moreover, our models considered other important potential
374 confounders such as age, body mass index, sex, and smoking, considering them
375 as modifying factors that affect the inflammatory biomarker concentration.[1, 2]

376 This study is not without limitations. First, due to lack of cut-offs established for
377 inflammatory biomarkers, we used median values age- and sex-adjusted. For IL-
378 6, we considered 1.9ng/L (1.9–6.95ng/L) for most age/sex group. Nevertheless,
379 our cut-offs are very close to those reported by the Asklepios Study (1.6ng/L for
380 IL-6),[50] where authors also found an association between DII score and IL-6. For
381 CRP, our cut-offs (0.11–0.79mg/dL) are close to that reported by Visser (0.22
382 mg/dL),[11] reporting a positive association with overweight in children and
383 adolescents. Second, we used inflammatory biomarkers considered non-
384 specificity in order to measure low-grade inflammation in healthy subjects;[1, 2]
385 however, we attempted to overcome this with adjusted models and the overall
386 inflammatory biomarker score. Third, we calculated the DII score using only 31
387 out of a possible 45 food parameters because only these components are present
388 in our database. Thus, DII score in our sample has a lower range (-5.36 to 4.02)
389 than the original possible ranges (-8.87 to 7.98).[23] However, it represents 56% of
390 the score range and is similar to the SEASONS cohort (57%).[26]

In summary, DII score was associated with IL-6, C4 and the overall inflammatory biomarker score after adjustments for biological and lifestyle characteristics. DII score was not associated with CRP and C3 in Portuguese adolescents.

DII score can be useful to assess the diet's inflammatory properties and its association with low-grade inflammation in adolescents.

ACKNOWLEDGEMENTS

The authors gratefully acknowledged the participation of all adolescents and their parents, teachers and schools of the LabMed and Physical Activity Study, the cooperation of volunteer's, the Department of Hygiene and Epidemiology (University of Porto) for the conversion food frequency questionnaire data into nutrients, and the Research Centre in Physical Activity, Health and Leisure (University of Porto) for the sponsoring the LabMed and Physical Activity Study.

CONFLICTS OF INTEREST

The authors declare: there are no conflicts of interest.

REFERENCES

1. Calder PC, Ahluwalia N, Albers R, Bosco N, Bourdet-Sicard R, Haller D *et al.* A consideration of biomarkers to be used for evaluation of inflammation in human nutritional studies. *The British journal of nutrition* 2013; **109 Suppl 1:** S1-34. e-pub ahead of print 2013/02/01; doi: 10.1017/s0007114512005119

2. Minihane AM, Vinoy S, Russell WR, Baka A, Roche HM, Tuohy KM *et al.* Low-grade inflammation, diet composition and health: current research evidence and its translation. *The British journal of nutrition* 2015; **114**(7)**:** 999-1012. e-pub ahead of print 2015/08/01; doi: 10.1017/s0007114515002093

3. van Greevenbroek MM, Schalkwijk CG, Stehouwer CD. Obesity-associated low-grade inflammation in type 2 diabetes mellitus: causes and consequences. *The Netherlands journal of medicine* 2013; **71**(4)**:** 174-187. e-pub ahead of print 2013/06/01;

4. Spranger J, Kroke A, Mohlig M, Hoffmann K, Bergmann MM, Ristow M *et al.* Inflammatory cytokines and the risk to develop type 2 diabetes: results of the prospective population-based European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam Study. *Diabetes* 2003; **52**(3)**:** 812-817. e-pub ahead of print 2003/02/28;

5. Berg AH, Scherer PE. Adipose tissue, inflammation, and cardiovascular disease. *Circulation research* 2005; **96**(9)**:** 939-949. e-pub ahead of print 2005/05/14; doi: 10.1161/01.res.0000163635.62927.34

6. Libby P. History of Discovery: Inflammation in Atherosclerosis. *Arteriosclerosis, thrombosis, and vascular biology* 2012; **32**(9)**:** 2045-2051. doi: 10.1161/ATVBAHA.108.179705

7. Fernandes JV, Cobucci RN, Jatoba CA, Fernandes TA, de Azevedo JW, de Araujo JM. The role of the mediators of inflammation in cancer development. *Pathology oncology research : POR* 2015; **21**(3)**:** 527-534. e-pub ahead of print 2015/03/06; doi: 10.1007/s12253-015-9913-z

8. Ostan R, Lanzarini C, Pini E, Scurti M, Vianello D, Bertarelli C *et al.* Inflammaging and cancer: a challenge for the Mediterranean diet. *Nutrients* 2015; **7**(4)**:** 2589-2621. e-pub ahead of print 2015/04/11; doi: 10.3390/nu7042589

9. Ford ES, Galuska DA, Gillespie C, Will JC, Giles WH, Dietz WH. C-reactive protein and body mass index in children: findings from the Third National Health and Nutrition Examination Survey, 1988-1994. *The Journal of pediatrics* 2001; **138**(4)**:** 486-492. e-pub ahead of print 2001/04/11; doi: 10.1067/mpd.2001.112898

10. Lambert M, Delvin EE, Paradis G, O'Loughlin J, Hanley JA, Levy E. C-reactive protein and features of the metabolic syndrome in a population-based sample of children and adolescents. *Clinical chemistry* 2004; **50**(10)**:** 1762-1768. e-pub ahead of print 2004/08/17; doi: 10.1373/clinchem.2004.036418

11. Visser M, Bouter LM, McQuillan GM, Wener MH, Harris TB. Low-grade systemic inflammation in overweight children. *Pediatrics* 2001; **107**(1)**:** E13. e-pub ahead of print 2001/01/03;

12. Warnberg J, Nova E, Moreno LA, Romeo J, Mesana MI, Ruiz JR *et al.* Inflammatory proteins are related to total and abdominal adiposity in a healthy adolescent population: the AVENA Study. *The American journal of clinical nutrition* 2006; **84**(3)**:** 505-512. e-pub ahead of print 2006/09/09;

13. Galcheva SV, Iotova VM, Yotov YT, Bernasconi S, Street ME. Circulating proinflammatory peptides related to abdominal adiposity and cardiometabolic risk factors in healthy prepubertal children. *European journal of endocrinology / European Federation of Endocrine Societies* 2011; **164**(4)**:** 553-558. e-pub ahead of print 2011/01/13; doi: 10.1530/eje-10-1124

14. Ford ES, Ajani UA, Mokdad AH. The metabolic syndrome and concentrations of C-reactive protein among U.S. youth. *Diabetes care* 2005; **28**(4)**:** 878-881. e-pub ahead of print 2005/03/29;

15. de Ferranti SD, Gauvreau K, Ludwig DS, Newburger JW, Rifai N. Inflammation and changes in metabolic syndrome abnormalities in US adolescents: findings from the 1988-1994 and 1999-2000 National Health and Nutrition Examination Surveys. *Clinical chemistry* 2006; **52**(7)**:** 1325-1330. e-pub ahead of print 2006/05/06; doi: 10.1373/clinchem.2006.067181

16. Jarvisalo MJ, Harmoinen A, Hakanen M, Paakkunainen U, Viikari J, Hartiala J *et al.* Elevated serum C-reactive protein levels and early arterial changes in healthy children. *Arteriosclerosis, thrombosis, and vascular biology* 2002; **22**(8)**:** 1323-1328. e-pub ahead of print 2002/08/13;

17. Juonala M, Magnussen CG, Berenson GS, Venn A, Burns TL, Sabin MA *et al.* Childhood adiposity, adult adiposity, and cardiovascular risk factors. *The New England journal of medicine* 2011; **365**(20)**:** 1876-1885. e-pub ahead of print 2011/11/18; doi: 10.1056/NEJMoa1010112

18. Zimmermann MB, Aeberli I. Dietary determinants of subclinical inflammation, dyslipidemia and components of the metabolic syndrome in overweight children: a review. *International journal of obesity (2005)* 2008; **32 Suppl 6:** S11-18. e-pub ahead of print 2008/12/23; doi: 10.1038/ijo.2008.202

19. Del Mar Bibiloni M, Maffeis C, Llompart I, Pons A, Tur JA. Dietary factors associated with subclinical inflammation among girls. *European journal of clinical nutrition* 2013; **67**(12)**:** 1264-1270. e-pub ahead of print 2013/10/24; doi: 10.1038/ejcn.2013.196

20. Aeberli I, Molinari L, Spinas G, Lehmann R, l'Allemand D, Zimmermann MB. Dietary intakes of fat and antioxidant vitamins are predictors of subclinical inflammation in overweight Swiss children. *The American journal of clinical nutrition* 2006; **84**(4)**:** 748-755. e-pub ahead of print 2006/10/07;

21. Barbaresko J, Koch M, Schulze MB, Nothlings U. Dietary pattern analysis and biomarkers of low-grade inflammation: a systematic literature review.

Nutrition reviews 2013; **71**(8)**:** 511-527. e-pub ahead of print 2013/07/20; doi: 10.1111/nure.12035

22. Cavicchia PP, Steck SE, Hurley TG, Hussey JR, Ma Y, Ockene IS *et al.* A new dietary inflammatory index predicts interval changes in serum high-sensitivity C-reactive protein. *The Journal of nutrition* 2009; **139**(12)**:** 2365-2372. e-pub ahead of print 2009/10/30; doi: 10.3945/jn.109.114025

23. Shivappa N, Steck SE, Hurley TG, Hussey JR, Hebert JR. Designing and developing a literature-derived, population-based dietary inflammatory index. *Public health nutrition* 2014; **17**(8)**:** 1689-1696. e-pub ahead of print 2013/08/15; doi: 10.1017/s1368980013002115

24. Xu H, Sjogren P, Arnlov J, Banerjee T, Cederholm T, Riserus U *et al.* A proinflammatory diet is associated with systemic inflammation and reduced kidney function in elderly adults. *The Journal of nutrition* 2015; **145**(4)**:** 729-735. e-pub ahead of print 2015/04/04; doi: 10.3945/jn.114.205187

25. van Woudenbergh GJ, Theofylaktopoulou D, Kuijsten A, Ferreira I, van Greevenbroek MM, van der Kallen CJ *et al.* Adapted dietary inflammatory index and its association with a summary score for low-grade inflammation and markers of glucose metabolism: the Cohort study on Diabetes and Atherosclerosis Maastricht (CODAM) and the Hoorn study. *The American journal of clinical nutrition* 2013; **98**(6)**:** 1533-1542. e-pub ahead of print 2013/10/25; doi: 10.3945/ajcn.112.056333

26. Shivappa N, Steck SE, Hurley TG, Hussey JR, Ma Y, Ockene IS *et al.* A population-based dietary inflammatory index predicts levels of C-reactive protein in the Seasonal Variation of Blood Cholesterol Study (SEASONS). *Public health nutrition* 2014; **17**(8)**:** 1825-1833. e-pub ahead of print 2013/10/11; doi: 10.1017/s1368980013002565

27. Tabung FK, Steck SE, Zhang J, Ma Y, Liese AD, Agalliu I *et al.* Construct validation of the dietary inflammatory index among postmenopausal women. *Annals of epidemiology* 2015. e-pub ahead of print 2015/04/23; doi: 10.1016/j.annepidem.2015.03.009

28. Shivappa N, Blair CK, Prizment AE, Jacobs DR, Jr., Steck SE, Hebert JR. Association between inflammatory potential of diet and mortality in the Iowa Women's Health study. *European journal of nutrition* 2015. e-pub ahead of print 2015/07/02; doi: 10.1007/s00394-015-0967-1

29. Shivappa N, Harris H, Wolk A, Hebert JR. Association between inflammatory potential of diet and mortality among women in the Swedish Mammography Cohort. *European journal of nutrition* 2015. e-pub ahead of print 2015/08/01; doi: 10.1007/s00394-015-1005-z

30. Galas A, Kulig J. Low-grade dietary-related inflammation and survival after colorectal cancer surgery. *Journal of cancer research and clinical oncology*

2014; **140**(9)**:** 1517-1525. e-pub ahead of print 2014/05/28; doi: 10.1007/s00432-014-1711-6

31. Wirth MD, Shivappa N, Steck SE, Hurley TG, Hebert JR. The dietary inflammatory index is associated with colorectal cancer in the National Institutes of Health-American Association of Retired Persons Diet and Health Study. *The British journal of nutrition* 2015**:** 1-9. e-pub ahead of print 2015/04/15; doi: 10.1017/s000711451500104x

32. Ruiz-Canela M, Zazpe I, Shivappa N, Hebert JR, Sanchez-Tainta A, Corella D *et al.* Dietary inflammatory index and anthropometric measures of obesity in a population sample at high cardiovascular risk from the PREDIMED (PREvencion con DIeta MEDiterranea) trial. *The British journal of nutrition* 2015; **113**(6)**:** 984-995. e-pub ahead of print 2015/02/28; doi: 10.1017/s0007114514004401

33. Garcia-Arellano A, Ramallal R, Ruiz-Canela M, Salas-Salvado J, Corella D, Shivappa N *et al.* Dietary Inflammatory Index and Incidence of Cardiovascular Disease in the PREDIMED Study. *Nutrients* 2015; **7**(6)**:** 4124-4138. e-pub ahead of print 2015/06/04; doi: 10.3390/nu7064124

34. Wirth MD, Burch J, Shivappa N, Violanti JM, Burchfiel CM, Fekedulegn D *et al.* Association of a dietary inflammatory index with inflammatory indices and metabolic syndrome among police officers. *Journal of occupational and environmental medicine / American College of Occupational and Environmental Medicine* 2014; **56**(9)**:** 986-989. e-pub ahead of print 2014/07/22; doi: 10.1097/jom.0000000000000213

35. Huh J, Riggs NR, Spruijt-Metz D, Chou CP, Huang Z, Pentz M. Identifying patterns of eating and physical activity in children: a latent class analysis of obesity risk. *Obesity (Silver Spring, Md.)* 2011; **19**(3)**:** 652-658. e-pub ahead of print 2010/10/12; doi: 10.1038/oby.2010.228

36. Rich C, Geraci M, Griffiths L, Sera F, Dezateux C, Cortina-Borja M. Quality Control Methods in Accelerometer Data Processing: Defining Minimum Wear Time. *PloS one* 2013; **8**(6)**:** e67206. doi: 10.1371/journal.pone.0067206

37. Trost SG, Loprinzi PD, Moore R, Pfeiffer KA. Comparison of accelerometer cut points for predicting activity intensity in youth. *Medicine and science in sports and exercise* 2011; **43**(7)**:** 1360-1368. e-pub ahead of print 2010/12/07; doi: 10.1249/MSS.0b013e318206476e

38. Lopes C, Aro A, Azevedo A, Ramos E, Barros H. Intake and adipose tissue composition of fatty acids and risk of myocardial infarction in a male Portuguese community sample. *Journal of the American Dietetic Association* 2007; **107**(2)**:** 276-286. e-pub ahead of print 2007/01/30; doi: 10.1016/j.jada.2006.11.008

39. Silva D, Rego C, Guerra A. Characterization of food habits and comparative study between two methods of food assessment in adolescents. *Rev Aliment Humana* 2004; **10:** 33–40.

40. Martins I, Porto A, Luísa O. *Table Composition of Foods*, National Institute of Health Dr Ricardo Jorge: Lisbon, Portugal, 2006.

41. Black AE. Critical evaluation of energy intake using the Goldberg cut-off for energy intake:basal metabolic rate. A practical guide to its calculation, use and limitations. *International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity* 2000; **24**(9)**:** 1119-1130. e-pub ahead of print 2000/10/18;

42. Trost SG, Ward DS, Moorehead SM, Watson PD, Riner W, Burke JR. Validity of the computer science and applications (CSA) activity monitor in children. *Medicine and science in sports and exercise* 1998; **30**(4)**:** 629-633. e-pub ahead of print 1998/05/05;

43. Cole TJ, Lobstein T. Extended international (IOTF) body mass index cut-offs for thinness, overweight and obesity. *Pediatric obesity* 2012; **7**(4)**:** 284-294. e-pub ahead of print 2012/06/21; doi: 10.1111/j.2047-6310.2012.00064.x

44. Tanner JM, Whitehouse RH. Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. *Archives of disease in childhood* 1976; **51**(3)**:** 170-179. e-pub ahead of print 1976/03/01;

45. Currie C, Molcho M, Boyce W, Holstein B, Torsheim T, Richter M. Researching health inequalities in adolescents: the development of the Health Behaviour in School-Aged Children (HBSC) family affluence scale. *Social science & medicine (1982)* 2008; **66**(6)**:** 1429-1436. e-pub ahead of print 2008/01/09; doi: 10.1016/j.socscimed.2007.11.024

46. World Health Organization. *Guidelines for Controlling and Monitoring the Tobacco Epidemic*, World Health Organization: Geneva, 1998.

47. Ridker PM, Rifai N, Stampfer MJ, Hennekens CH. Plasma concentration of interleukin-6 and the risk of future myocardial infarction among apparently healthy men. *Circulation* 2000; **101**(15)**:** 1767-1772. e-pub ahead of print 2000/04/19;

48. Cesari M, Penninx BW, Newman AB, Kritchevsky SB, Nicklas BJ, Sutton-Tyrrell K *et al.* Inflammatory markers and onset of cardiovascular events: results from the Health ABC study. *Circulation* 2003; **108**(19)**:** 2317-2322. e-pub ahead of print 2003/10/22; doi: 10.1161/01.cir.0000097109.90783.fc

49. Tremblay MS, Colley RC, Saunders TJ, Healy GN, Owen N. Physiological and health implications of a sedentary lifestyle. *Applied physiology, nutrition, and metabolism = Physiologie appliquee, nutrition et metabolisme* 2010; **35**(6)**:** 725-740. e-pub ahead of print 2010/12/18; doi: 10.1139/h10-079

50. Shivappa N, Hebert JR, Rietzschel ER, De Buyzere ML, Langlois M, Debruyne E *et al.* Associations between dietary inflammatory index and

inflammatory markers in the Asklepios Study. *The British journal of nutrition* 2015; **113**(4)**:** 665-671. e-pub ahead of print 2015/02/03; doi: 10.1017/s000711451400395x

FIGURE LEGENDS

Table 1: Mean, standard deviation and correlation with final score of food parameters included in the calculation of DII score for the adolescents from LabMed Physical Activity Study

Abbreviations: DII – Dietary Inflammatory Index; RE = retinol equivalents; r_s – Spearman correlation coefficient.

[a] Correlation coefficients based on Spearman test, $p<0.001$ for all.

Table 2: Participants' characteristics according to sex in Portuguese adolescents from LabMed Physical Activity Study

Abbreviations: DII – Dietary inflammatory index, CRP – C-reactive protein, IL-6 – Interleukin-6, C3 – Complement component 3, C4 – Complement component 4.

[a] The data shown in percentage for categorical variables and median (interquartile range) for continuous variables.

[b] P value was calculated based on Qui-squared test for categorical variables and Mann–Whitney U test for continuous variables.

[c] Qui-squared test performed with "Current smokers" and "Occasional smokers" together to improve power of test.

[d] It was considered n=329 (55.9% girls), because there is some missing values in Sedentary time and Moderate-to-vigorous physical activity variables.

[e] Qui-squared test performed with "Current smokers" and "Occasional smokers" together to improve power of test.

[f] Overall inflammatory biomarker score were designed summing the inflammatory biomarkers (CRP, IL-6, C3 and C4) categories, wherein for each category was assigned one point if the biomarker was above the median adjusted by age and sex or zero if below the median.

Table 3: Differences and correlations between inflammatory biomarkers and DII score in Portuguese adolescents from the LabMed Physical Activity Study

Abbreviations: DII – Dietary inflammatory index, CRP – C-reactive protein, IL-6 – Interleukin-6, C3 – Complement component 3, C4 – Complement component 4.

[a] P values and rs coefficients were based on Spearman test.

[b] P value was based on Qui-square test.

[c] Overall inflammatory biomarker score were designed summing the inflammatory biomarkers (CRP, IL-6, C3 and C4) categories, wherein for each category was assigned one point if the biomarker was above the median adjusted by age and sex or zero if below the median.

Table 4: Association between DII score tertiles and inflammatory biomarkers categories among adolescents from LabMed Physical Activity Study

Abbreviations: OR – odds ratio, CI – confidence interval, DII – Dietary inflammatory index, CRP – C-reactive protein, IL-6 – Interleukin-6, C3 – Complement component 3, C4 – Complement component 4

[a] All fully adjusted model were adjusted for Sex, Age, Pubertal stage – Tanner A and B, Body mass index, Energy intake, Socio-economic status, Sedentary behaviour, Moderate-to-vigorous physical activity, and Smoking habits. There are some missing values in Sedentary time and Moderate-to-vigorous physical activity variables, so this models is based in n=329 (55.9% girls).

[b] Overall inflammatory biomarker score were designed summing the inflammatory biomarkers (CRP, IL-6, C3 and C4) categories, wherein for each category was assigned one point if the biomarker was above the median adjusted by age and sex or zero if below the median.

Table 1: Mean, standard deviation and correlation with final score of food parameters included in the calculation of DII score for adolescents from LabMed Physical Activity Study

DII food parameters	Mean	SD	r_s[a]
Alcohol (g/day)	1.22	3.94	-0,219
Vitamin B12 (µg/day)	13.60	10.80	-0,551
Vitamin B6 (mg/day)	2.38	0.88	-0,904
β-Carotene (µg/day)	1 011.43	902.78	-0,699
Caffeine (g/day)	33.51	34.71	-0,185
Carbohydrate (g/day)	266.47	97.26	-0,745
Cholesterol (mg/day)	357.56	168.84	-0,595
Energy (kcal/day)	2 127.72	680.98	-0,809
Total fat (g/day)	76.65	27.04	-0,708
Fiber (g/day)	21.85	10.18	-0,903
Folic acid (µg/day)	372.69	182.23	-0,893
Green/black tea (g/day)	17.20	42.51	-0,263
Iron (mg/day)	16.63	6.33	-0,885
Magnesium (mg/day)	331.71	119.47	-0,919
Monounsaturated fatty acids (g/day)	31.49	11.74	-0,717
Niacin (mg/day)	24.91	9.08	-0,840
n-3 Fatty acids (g/day)	1.40	0.61	-0,793
n-6 Fatty acids (g/day)	10.33	4.67	-0,680
Onion (g/day)	13.27	21.63	-0,409
Protein (g/day)	99.92	34.77	-0,794
Polyunsaturated fatty acids (g/day)	13.97	5.73	-0,727
Riboflavin (mg/day)	2.48	1.00	-0,726
Saturated fat (g/day)	24.60	8.84	-0,580
Selenium (mg/day)	102.87	39.99	-0,827
Thiamin (mg/day)	1.79	0.64	-0,859
Trans fat (g/day)	1.06	0.59	-0,455
Vitamin A (RE/day)	1 237.95	1 277.34	-0,735
Vitamin C (mg/day)	148.70	97.01	-0,817
Vitamin D (µg/day)	4.86	2.89	-0,645
Vitamin E (mg/day)	8.91	4.07	-0,860
Zinc (mg/day)	13.10	4.72	-0,703

Abbreviations: DII – Dietary Inflammatory Index; RE – retinol equivalents; SD – standard deviation; r_s – correlation coefficient.

[a] Correlation coefficients based on Spearman Test. All $p<0.001$.

Table 2: Participants' characteristics according to sex in adolescents from the LabMed Physical Activity Study

		All [a] (n=412)	Girls [a] (n=216)	Boys [a] (n=196)	p [b]
DII score		0.61 (-1.10 – 2.10)	0.84 (-0.82 – 2.27)	0.43 (-1.60 – 1.89)	**0.032**
Age (years)		14.9 (12.6 – 15.7)	14.9 (12.6 – 15.6)	15.0 (12.7 – 15.8)	0.143
Pubertal stage: Tanner A [c]	2	7.2%	33.3%	11.7%	**0.001**
	3	33.0%	54.6%	36.2%	
	4	47.1%	12.0%	38.8%	
	5	12.6%	2.8%	13.3%	
Pubertal stage: Tanner B [c]	2	7.0%	22.7%	11.7%	**<0.001**
	3	20.6%	46.8%	18.4%	
	4	50.2%	27.8%	54.1%	
	5	22.1%	3.2%	15.8%	
Body mass index	underweight	3.6%	3.7%	3.6%	0.574
	normal weight	66.7%	65.3%	68.4%	
	overweight	22.1%	21.8%	22.4%	
	obese	7.5%	9.3%	5.6%	
Socio-economic status		6.0 (5.0 – 8.0)	6.0 (6.0 – 8.0)	6.0 (5.0 – 8.0)	0.416
Energy intake	(kj.day^{-1})	8 636 (6 689 – 10 854)	8 439 (6 526 – 10 063)	8 887 (6 899 – 11 428)	**0.005**
	(kcal.day^{-1})	2 063 (1 598 – 2 593)	2 016 (1 559 – 2 404)	2 123 (1 648 – 2 730)	
Sedentary behaviour (minutes.day^{-1}) [d]		667.4 (619.4 – 725.3)	678.4 (632.8 – 734.1)	645.9 (607.5 – 713.2)	**0.003**
Moderate-to-vigorous physical activity (minutes.day^{-1}) [d]		51.0 (39.1 – 65.3)	45.5 (35.1 – 59.5)	56.7 (43.0 – 71.5)	**<0.001**

Smoking habits [e]	Current smokers	1.0%	0.9%	1.0%	**0.037**
	Occasional smokers	1.7%	1.4%	2.0%	
	Former smokers	7.8%	4.6%	11.2%	
	Non-smokers	89.6%	93.1%	85.7%	
CRP (mg/L)		0.20 (0.11 – 0.77)	0.11 (0.11 – 0.49)	0.34 (0.11 – 1.26)	**<0.001**
IL-6 (ng/L)		1.90 (1.90 – 3.40)	1.90 (1.90 – 3.40)	1.90 (1.90 – 3.35)	0.561
C3 (mg/dL)		116.0 (107.0 – 126.5)	119.0 (107.0 – 127.0)	115.0 (106.5 – 126.0)	0.888
C4 (mg/dL)		20.0 (16.0 – 24.0)	20.0 (16.0 – 25.0)	20.0 (17.0 – 24.0)	0.561
Overall inflammatory biomarker score [f]		-0.55 (-1.77 – 1.41)	-0.51 (-1.75 – 1.30)	-0.59 (-1.84 – 1. 43)	0.844

Abbreviations: DII – Dietary inflammatory index, CRP – C-reactive protein, IL-6 – Interleukin-6, C3 - Complement component 3, C4 - Complement component 4.

[a] The data shown in percentage for categorical variables and median (interquartile range) for continuous variables.

[b] P value was calculated based on Qui-squared test for categorical variables and Mann–Whitney U test for continuous variables.

[c] Tanner A indicates development stages of breast in girls and genitalia (penis size and testicular volume) in boys; Tanner B indicates development stages of public hair distribution (Tanner B).

[d] It was considered n=329 (55.9% girls), because there is some missing values in Sedentary time and Moderate-to-vigorous physical activity variables.

[e] Qui-squared test performed with "Current smokers" and "Occasional smokers" together to improve power of test.

[f] Overall inflammatory biomarker score were designed calculating an age and gender adjusted z-score for each inflammatory biomarker (CRP, IL-6, C3 and C4) and summing them.

Table 3: Differences and correlations between inflammatory biomarkers and DII score in adolescents from the LabMed Physical Activity Study

			DII score					
			Continuous		Tertiles - inflammatory properties			
			r_s	p [a]	1st - Low (< -1.34)	2nd - Medium (-1.34 to 1.41)	3rd - High (>1.41)	p [b]
CRP	Continuos		- 0.058	0.240				
	Categories	Lower			58.5%	51.5%	53.7%	0.547
		Higher			41.5%	48.5%	46.3%	
IL-6	Continuos		0.107	**0.031**				
	Categories	Lower			69.1%	69.2%	54.4%	**0.011**
		Higher			30.9%	30.8%	45.6%	
C3	Continuos		0.034	0.489				
	Categories	Lower			45.7%	55.6%	51.0%	0.301
		Higher			54.3%	44.4%	49.0%	
C4	Continuos		0.005	0.924				
	Categories	Lower			54.3%	56.8%	47.0%	0.203
		Higher			45.7%	43.2%	53.0%	
Overall inflammatory biomarker score [c]	Continuos		0.031	0.528				
	Categories	Lower			48.9%	47.9%	38.9%	0.183
		Higher			51.1%	52.1%	61.1%	

Abbreviations: DII – Dietary inflammatory index, CRP – C-reactive protein, IL-6 – Interleukin-6, C3 – Complement component 3, C4 – Complement component 4.

a P values and r_s coefficients were based on Spearman test.

b P values were based on Qui-square test.

c Overall inflammatory biomarker score were designed summing the inflammatory biomarkers (CRP, IL-6, C3 and C4) categories, wherein for each category was assigned one point if the biomarker was above the median adjusted by age and sex or zero if below the median.

Table 4: Association between DII score tertiles and inflammatory biomarkers categories among adolescents from LabMed Physical Activity Study

	DII score: OR (95% CI)			
	1st Tertile Low (< -1.34)	2nd Tertile Medium (-1.34 to 1.41)	3rd Tertile High (>1.41)	P_{trend}
CRP models				
Crude	1.00	1.33 (0.80 – 2.21)	1.22 (0.72 – 2.05)	0.548
Sex-adjusted	1.00	1.35 (0.81 – 2.25)	1.26 (0.74 – 2.13)	0.512
Fully-adjusted[a]	1.00	1.71 (0.83 – 3.51)	2.33 (0.88 – 6.20)	0.230
IL-6 models				
Crude	1.00	1.00 (0.58 – 1.72)	**1.88 (1.09 – 3.24)**	**0.011**
Sex-adjusted	1.00	0.99 (0.57 – 1.71)	**1.84 (1.06 – 3.18)**	**0.015**
Fully-adjusted[a]	1.00	1.44 (0.68 – 3.08)	**3.38 (1.24 – 9.20)**	**0.023**
C3 models				
Crude	1.00	0.67 (0.41 – 1.12)	0.81 (0.48 – 1.36)	0.302
Sex-adjusted	1.00	0.67 (0.40 – 1.11)	0.80 (0.47 – 1.35)	0.296
Fully-adjusted[a]	1.00	0.75 (0.36 – 1.57)	1.71 (0.63 – 4.66)	**0.044**
C4 models				
Crude	1.00	0.90 (0.54 – 1.50)	1.34 (0.80 – 2.25)	0.204
Sex-adjusted	1.00	0.91 (0.54 – 1.50)	1.35 (0.80 – 2.27)	0.199
Fully-adjusted[a]	1.00	1.13 (0.57 – 2.28)	**3.12 (1.21 – 8.10)**	**0.016**
Overall inflammatory biomarker score models[b]				
Crude	1.00	1.04 (0.63 – 1.72)	1.50 (0.89 – 2.53)	0.184
Sex-adjusted	1.00	1.04 (0.63 – 1.73)	1.51 (0.89 – 2.55)	0.188
Fully-adjusted[a]	1.00	1.75 (0.84 – 3.66)	**5.61 (2.00 – 15.78)**	**0.002**

Abbreviations: OR – odds ratio, CI – confidence interval, DII – Dietary inflammatory index, CRP – C-reactive protein, IL-6 – Interleukin-6, C3 – Complement component 3, C4 – Complement component 4.
a All fully adjusted model were adjusted for Sex, Age, Pubertal stage – Tanner A and B, Body mass index, Energy intake, Socio-economic status, Sedentary behaviour, Moderate-to-vigorous physical activity, and Smoking habits. There are some missing values in Sedentary time and Moderate-to-vigorous physical activity variables, so this models is based in n=329 (55.9% girls).
b Overall inflammatory biomarker score were designed summing the inflammatory biomarkers (CRP, IL-6, C3 and C4) categories, wherein for each category was assigned one point if the biomarker was above the median adjusted by age and sex or zero if below the median.

8. General discussion

The main findings of this thesis suggest that nutrients, foods, and dietary patterns may influence low-grade inflammation in adolescents, particularly: high intake of SFA, which acted as a pro-inflammatory factor high consumption of n-3 PUFA, namely ALA and EPA+DHA, vegetable soup at least twice a day, adherence to the 5-a-day recommendation, and eating a high variety of vegetables, all of which acted as anti-inflammatory factors. In addition, a high-inflammatory dietary pattern in adolescents increased the probability of low-grade inflammation in adolescents. These results were independent of sex, age, physical activity (moderate-to-vigorous and sedentary time), body mass index, pubertal development, socioeconomic status, smoking habits, and total energy intake.

To the best of our knowledge, no prior study has specifically analyzed n-3 and n-6 PUFA, such as AA, LA, ALA, EPA, and DHA, as predictors of low-grade inflammation in apparently healthy adolescents. Furthermore, no previous publication has specifically reported any association between low-grade inflammation and fruit and vegetable intake based on adherence to the 5-a-day recommendation. In addition, no authors have yet described a relationship between vegetable variety and low-grade inflammation in adolescents. Finally, this is the first study exploring associations between the DII score and inflammatory biomarkers in adolescents.

Taken together, these results emphasize the importance of a prudent dietary pattern,[344] which is a balanced diet characterized by high consumption of vegetables, legumes, whole-grain cereal, fish, fruit, and nuts; and which may provide significant sources of fiber, minerals, vitamins, flavonoids, other antioxidants, and PUFA, and minimal amount of SFA.

In paper I, we showed that a SFA daily intake higher than 11.1% of total energy intake doubled the odds of having a higher level of IL-6 and a higher overall inflammatory biomarkers score. No associations were found for CRP, C3, or C4. In accordance with our findings, Arya et al.[201] found no association between SFA and CRP. Aeberli et al.[166] did not find an association between SFA and IL-6, but did for CRP, in children and adolescents, conflicting with our findings. When considering serum lipids, Kalogeropoulos et al.,[345] in accordance with our

findings, reported that IL-6 had a positive correlation with concentration of plasma SFA in healthy adults; however, Wang et al.,[202] in conflict with our findings, found that IL-6 had a negative relationship with serum levels of some SFA, such as pentadecanoic and heptadecanoic acids, in adolescents.

We have also suggested in paper I that ALA and EPA+DHA daily consumption of 0.50% and 0.22% of total energy intake or more, respectively, significantly reduced (by half) the odds of having a higher overall inflammatory biomarker score, although no association has been found for the inflammatory biomarkers individually. In contrast, Aeberli et al.[166] reported a positive relationship between PUFA and CRP in children and adolescents. However, these results are difficult to interpret without considering n-3 and n-6 separately, since n-6 PUFA can increase inflammation and are partly inhibited by n-3 PUFA.[205] Data from adult-based studies showed that dietary intakes of ALA,[346] EPA and DHA[347, 348] were inversely associated with CRP levels.

Our study agreed with literature findings regarding the protective role of fruit and vegetable intake on low-grade inflammation,[69] highlighting the public health message—known worldwide as the 5-a-day recommendation—which encourages individuals to consume five or more daily servings of fruits and vegetables. Although no relationship was demonstrated when fruits and vegetables were treated individually, our paper II showed that adherence to the 5-a-day was strongly and inversely associated with low-grade inflammation, being associated with four in five inflammation measures: namely CRP, IL-6, C4, and the overall inflammatory biomarker score. Our results aligned with Holt et al.[214] which also showed an inverse association between fruit and vegetable intake and CRP and IL-6 in adolescents. The novelty of our data also includes the fact that no previous study has demonstrated a relationship between the consumption of fruits and vegetables and C4 or a score of inflammation.

Furthermore, our paper II also identified that daily consumption of less than 2 portions of vegetable soup increased by about 13 times the likelihood of having higher IL-6. To our knowledge, no other observational study found a relationship between vegetable soup and IL-6. However, one experimental study[349] found a relationship between vegetable soup and CRP, but two observational[250, 252]

studies did not. This finding has particular importance for Portuguese individuals because vegetable soup, together with lettuce and tomato, represents almost 50% of the weekly mean consumption of vegetables in adults.[350] These results gives new reason to maintain and increase this habit, in order to improve nutritional intake and counteract low-grade inflammation, especially in adolescents, since their frequency of vegetable soup consumption may be considered low in the present study as also described by other authors.[94]

In addition, in our paper III, ours results indicated that a high variety of vegetable intake is inversely associated with CRP, independent of vegetable quantity consumed. According to our knowledge, no previous study found an association between vegetable variety and low-grade inflammation, but Bhupathiraju and Tucker found an association between low fruit and vegetable variety and high level of CRP.[243]

In this context, adolescents who adhere to the 5-a-day recommendation, consume vegetable soup at least twice a day, or eat a wide variety of vegetables may correspondingly consume more relevant nutrients to prevent a low-grade inflammation state, compared to adolescents who do not follow this diet. In fact, dietary fiber,[66, 207] antioxidant vitamins, carotenoids,[299] and flavonoid-rich foods[218] have been identified as having an anti-inflammatory effect; authors showed that dietary fiber,[213] vitamin A,[215, 223] vitamin C,[166, 214] vitamin E,[166, 223] β-carotene,[166, 214] flavonoid,[214] selenium,[223] and magnesium[232-234] were inversely associated with low-grade inflammation in children[166, 215, 234] and adolescents.[166, 214, 223, 232-234] Furthermore, a diet characterized by a wide variety of vegetables may favor reduced exposure to any undesirable, harmful components,[351] and helps individuals to consume a larger number of nutrients and bio-active components,[81, 352] [219] which may exert a synergistic effect and further reduce inflammation.[353]

According to papers II and III, we found an association between low-grade inflammation, namely CRP, and variety of vegetable intake, but we did not find the same association with vegetable quantity intake. The association between the quantity of vegetables consumed and CRP in a cross-sectional analysis seems to be controversial; on one hand, some researchers[244, 246, 250, 251] reported this

relationship for adults,[246, 250, 251] children and adolescents;[244] on the other hand, other researchers[214, 245, 247] did not for adults[245, 247] nor adolescents.[214] However, in a cross-sectional study of adults,[243] Bhupathiraju and Tucker found that fruit and vegetable variety (in a single variable) appears to be important in CRP.

The limitation of detecting the effect of a single component in healthy outcomes is recognized. However, the cumulative effects of multiple components included in a dietary pattern may be sufficiently large to be detectable. In addition, dietary patterns consider synergistic or antagonistic interactions among food components that nutrients or foods may not measure properly.[274]

In accordance, we showed in paper IV that DII score strongly predicted low-grade inflammation, being associated with 3 of the 5 inflammation measures. Adolescents whose diets showed low- or medium-inflammatory properties had a lower prevalence of higher IL-6, C4 and the overall inflammatory biomarker score. These findings are in accordance with other authors showing a relationship between DII score and IL-6 and an inflammatory biomarker score.[301, 303] Our study innovates in showing the association between DII and C4.

Dietary fiber, phytochemicals such as flavonoids and carotenoids, vitamins such as A, C, and E, minerals such as magnesium and zinc, and n-3 PUFAs are the food components that most contribute to a lower DII score (an anti-inflammatory dietary pattern), while total fat, SFA, and TFA are nutrients that most contribute to a higher DII score (a pro-inflammatory dietary pattern).[299] Although the DII score is a literature-based tool developed based on the role of diet in inflammatory biomarkers,[298, 299] it effectively captures the concept of a prudent diet[344] to prevent inflammation. In fact, diets based on Mediterranean and macrobiotic dietary styles produced strong anti-inflammatory DII scores, while fast food dietary styles produced a strong pro-inflammatory DII score.[325]

Another consideration in our study is the finding that the relationships between dietary and nutritional intakes and the overall inflammatory biomarker score was stronger than any single biomarker in showing a link between fatty-acid intakes, the adherence to the 5-a-day recommendation, and the DII score. In fact,

inflammatory biomarkers in general are considered non-specific pro-inflammatory response markers in healthy people, and the biomarkers' signatures that best represent low-grade inflammation are yet to be fully understood.[70] Thus, the overall inflammatory biomarker score was shown to be a more complex and integrated assessment of low-grade inflammation than just an inflammatory biomarker alone. This score considers who is above the median adjusted for age and sex, taking into account the effects of all inflammatory biomarkers—including those that were shown to have a relationship with dietary and nutritional intake and those that were not—and seems to better represent low-grade inflammation in this group of adolescents.

The difficulty to study the relationship between dietary and nutritional intakes and inflammatory biomarkers in healthy young populations was previously recognized.[10] Thus, this overall inflammatory biomarker score may add more one possibility to overcome the limitation. Other authors have used other inflammatory scores, with different biomarker combinations, to measure low-grade inflammation. In a longitudinal study of adults, van Bussel et al.[242] found that some healthy diet parameters were associated with less low-grade inflammation; Tabung et al.[303] and van Woudenbergh et al.[301] found the DII score was positively associated with low-grade inflammation in adults. To the best of our knowledge, no other prior study has shown a relationship between specifics fatty-acid intake or the adherence to the 5-a-day recommendation and an inflammatory score.

Following the overall inflammatory biomarker score, IL-6 was the inflammatory biomarker most associated with nutritional and dietary intake—namely with SFA, the 5-a-day recommendation, vegetable soup, and the DII score. IL-6 is an important factor for acute phase-protein hepatic synthesis[100] such as CRP, C3 and C4; therefore, it can be argued that in a young and healthy population, increase of IL-6 can be the first manifestation of low-grade inflammation. In addition, the inclusion of C3 and C4 in this study was an innovation. C4 shows to be sensitive to dietary and nutritional variables, in particular to the 5-a-day recommendation and to DII score, and it was a novelty for the studies regarding

the relationship between dietary and nutritional intake and low-grade inflammation.

The strength of this study, beyond the novelty of our aims, includes the use of objective physical activity measurements (moderate-to-vigorous and sedentary time) as covariates, since physical activity is an important inductor of an anti-inflammatory environment,[174] and sedentary time is a risk factor for cardiovascular health.[172] In addition, we only used the accurate food-frequency questionnaires, according to Goldberg's method,[333] to reduce the bias of misreporting. Moreover, we included important biological and lifestyle variables such as age,[104, 105] body mass index,[19-21] sex,[181] smoking,[173] and socioeconomic status[54-56] considered important modifiers of inflammatory biomarkers concentration.[70, 100]

Other strengths of our study are the evaluation of the C3 and C4 biomarkers—the roles of which in the relationship between diet and inflammation are still not well known—and the use of a set of inflammatory biomarkers (rather than just one), as well as the calculation of an inflammatory biomarker score, a more complex and integrated assessment of low-grade inflammation. This approach seems to be sensible to others authors[242, 301, 303, 326] studying the relationship between low-grade inflammation and some aspects of dietary and nutritional intake. Considering that inflammatory biomarkers, in general and in healthy people, are nonspecific pro-inflammatory response markers,[70] our score attempted to combine the effects of all inflammatory biomarkers analyzed. Furthermore, studying the associations between dietary intake and inflammatory biomarkers in a healthy young population is considered a challenge,[10] so this inflammatory score makes it easier to overcome this difficulty.

This study has some limitations that should be acknowledged. First, in regard to our instrument to measure dietary and nutritional intake, we used a food-frequency questionnaire that may overestimate the food intake.[353] To overcome this limitation, we excluded 150 participants who were considered misreports based on the Goldberg method[333]. In addition, this questionnaire combines several fruits and vegetables into single items; thus, the variety may have been

underestimated. Furthermore, several studies used food-frequency questionnaires to measure variety of fruit and vegetable variety,[243, 354-356] which may be a positive factor for comparison studies. Conversely, the food-frequency questionnaire is a good instrument to rank subjects by their level of consumption,[357] so we divided responses into three categories of dietary and nutritional variables by using tertile cut-offs, such as fatty acids consumption, intake of fruit variety and vegetable variety, and DII score, or by using the 5-a-day recommendation cut-offs for consumption of fruits and vegetables.

Second, the variety intake has been correlated with increased quantity intake,[358] and this can be another limitation when studying fruit and vegetable variety. However, in the fully adjusted models (model adjusted 3), we included intake information, and found that the results changed for overall inflammatory biomarker score model.

Third, we calculated the DII score using only 31 out of a possible 45 food parameters, because only 31 components were present in our database. Thus, the DII score in our sample has a lower range (-5.36 to 4.02) than the original possible ranges (-8.87 to 7.98),[299] but a similar score range was reached by a validation study of DII score.[302]

Finally, because of the lack of established cut-off points for inflammatory biomarkers for adolescents,[10] we used sex-age-adjusted median values to create two categories of inflammatory biomarkers. It was important to identify subjects with higher inflammatory biomarker level, since the increase of inflammatory biomarkers in low-grade inflammation may be very low or even absent,[100] as well as to ensure an adequate statistical power (in our study was always high than 0.80), since it is a difficulty recognized when examining the relationship between dietary intakes and inflammatory biomarkers in healthy populations.[359] This methodology allowed us to ranks the sample and classify it according to lowest and highest inflammatory state. Nevertheless, our cut-off points for IL-6 (1.9ng/L for most age/sex groups, but varying from 1.9 to 6.95ng/L) are very similar to the ones reported in the Asklepios Study (1.6ng/L),[360] and the authors found an association with the DII score. In addition, our cut-off points for CRP (varying from 0.11 to 0.79mg/dL) are close

to the ones reported by Visser et al. (0.22mg/dL),[21] which showed a positive association between low-grade inflammation and higher weight in children and adolescents.

9. Conclusions

Major conclusions of these studies were:

- Fatty-acids intake is related to a low-grade inflammation state in adolescents; SFA was positively associated with IL-6 and the overall inflammatory biomarkers score, and ALA and EPA+DHA were inversely associated with the overall inflammatory biomarkers score, independent of biological and lifestyle variables like body mass index, age, gender, pubertal stage, physical activity, sedentary time, socioeconomic status, and smoking habits. The other dietary fatty acids studied (MUFA, AA, LA, and TFA) were not predictive of low-grade inflammation;
- Vegetable soup intake was inversely associated with IL-6 after adjustments for several biological and lifestyle confounders. Fruit or vegetable intakes, considered individually, were not associated with any inflammatory biomarker or overall inflammatory biomarker score. Five or more portions of fruit and vegetable per day (the adherence to the 5-a-day recommendation), when compared with less than one portions per day, were inversely associated with CRP, IL-6, C4 and the overall inflammatory biomarker score after adjustments for biological and lifestyle characteristics;
- Vegetable variety intake, independent of vegetable quantity intake, was inversely associated with CRP after adjustments for several biological and lifestyle confounders. Variety related to fruit intake remained unclear;
- DII score was associated with IL-6, C4, and the overall inflammatory biomarker score after adjustments for biological and lifestyle characteristics. DII score was not associated with CRP and C3 in adolescents.

Taken together, in order to prevent a low-grade inflammation state in adolescents, these findings support 1) a high intake of n-3 PUFA, that is, ALA and EPA+DHA, and a low consumption of SFA; 2) the consumption of vegetable soup twice a day; 3) adherence to the 5-a-day recommendation; and 4) the guideline for choosing a wide variety of vegetables. In addition, DII score can be useful to

assess the diet's inflammatory properties and its association with low-grade inflammation in adolescents.

Future studies about the relationship between low-grade inflammation and other nutrients, foods, and dietary pattern — such as carbohydrate intake or "fast-food" consumption and Mediterranean diet — in adolescents could be interesting. In addition, DII score could be future studied in its relations regarding to inflammatory biomarkers and with disease risk factors in adolescents, using longitudinal designs. Furthermore, intervention studies to increase the consumption of ALA, EPA and DHA, to decrease the intake of SFA, promoting the adherence to 5-a-day recommendation, and stimulating vegetable soup twice a day may be conducted in adolescents.

10. References

1. Patton GC, Viner R. Pubertal transitions in health. Lancet (London, England). 2007; 369(9567):1130-9.

2. Patton GC, Sawyer SM, Santelli JS, Ross DA, Afifi R, Allen NB, et al. Our future: a Lancet commission on adolescent health and wellbeing. Lancet (London, England). 2016

3. World Health Organization. Health for the World's Adolescents: A second chance in the second decade. Geneva, Switzerland: World Health Organization; 2014. Disponível em: http://apps.who.int/adolescent/second-decade/.

4. Mahan LK, Escott-Stump S, Raymond JL. Krause's food & the nutrition care process. 13th ed.: Elsevier Health Sciences; 2012.

5. Wardle J. Parental influences on children's diets. The Proceedings of the Nutrition Society. 1995; 54(3):747-58.

6. Lake AA, Mathers JC, Rugg-Gunn AJ, Adamson AJ. Longitudinal change in food habits between adolescence (11-12 years) and adulthood (32-33 years): the ASH30 Study. Journal of public health (Oxford, England). 2006; 28(1):10-6.

7. Global Burden of Disease Pediatrics C. Global and national burden of diseases and injuries among children and adolescents between 1990 and 2013: Findings from the global burden of disease 2013 study. JAMA Pediatrics. 2016; 170(3):267-87.

8. Portugal Statistics. Complete Mortality Tables for Portugal. 2016. [atualizado em: May 27, 2016]. Disponível em: https://www.ine.pt/xportal/xmain?xpid=INE&xpgid=ine_indicadores&indOcorrCod=0004159&contexto=bd&selTab=tab2, .

9. Gore FM, Bloem PJ, Patton GC, Ferguson J, Joseph V, Coffey C, et al. Global burden of disease in young people aged 10–24 years: a systematic analysis. The Lancet. 2011; 377(9783):2093-102.

10. Warnberg J, Nova E, Romeo J, Moreno LA, Sjostrom M, Marcos A. Lifestyle-related determinants of inflammation in adolescence. The British journal of nutrition. 2007; 98 Suppl 1:S116-20.

11. Sawyer SM, Afifi RA, Bearinger LH, Blakemore SJ, Dick B, Ezeh AC, et al. Adolescence: a foundation for future health. Lancet (London, England). 2012; 379(9826):1630-40.

12. Labayen I, Ortega FB, Sjostrom M, Ruiz JR. Early life origins of low-grade inflammation and atherosclerosis risk in children and adolescents. The Journal of pediatrics. 2009; 155(5):673-7.

13. van Greevenbroek MM, Schalkwijk CG, Stehouwer CD. Obesity-associated low-grade inflammation in type 2 diabetes mellitus: causes and consequences. The Netherlands journal of medicine. 2013; 71(4):174-87.

14. Spranger J, Kroke A, Mohlig M, Hoffmann K, Bergmann MM, Ristow M, et al. Inflammatory cytokines and the risk to develop type 2 diabetes: results of the prospective population-based European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam Study. Diabetes. 2003; 52(3):812-7.

15. Uauy R, Corvalan C, Casanello P, Kuzanovic J. Intervention strategies for preventing low birthweight in developing countries: importance of considering multiple interactive factors. 2013

16. Berg AH, Scherer PE. Adipose tissue, inflammation, and cardiovascular disease. Circulation research. 2005; 96(9):939-49.

17. Libby P, Ridker PM, Hansson GK. Inflammation in Atherosclerosis: From Pathophysiology to Practice. Journal of the American College of Cardiology. 2009; 54(23):2129-38.

18. Libby P. History of Discovery: Inflammation in Atherosclerosis. Arteriosclerosis, thrombosis, and vascular biology. 2012; 32(9):2045-51.

19. Ford ES, Galuska DA, Gillespie C, Will JC, Giles WH, Dietz WH. C-reactive protein and body mass index in children: findings from the Third National Health and Nutrition Examination Survey, 1988-1994. The Journal of pediatrics. 2001; 138(4):486-92.

20. Lambert M, Delvin EE, Paradis G, O'Loughlin J, Hanley JA, Levy E. C-reactive protein and features of the metabolic syndrome in a population-based sample of children and adolescents. Clinical chemistry. 2004; 50(10):1762-8.

21. Visser M, Bouter LM, McQuillan GM, Wener MH, Harris TB. Low-grade systemic inflammation in overweight children. Pediatrics. 2001; 107(1):E13.

22. Olds T, Maher C, Zumin S, Peneau S, Lioret S, Castetbon K, et al. Evidence that the prevalence of childhood overweight is plateauing: data from nine countries. International journal of pediatric obesity : IJPO : an official journal of the International Association for the Study of Obesity. 2011; 6(5-6):342-60.

23. Wijnhoven TM, van Raaij JM, Spinelli A, Starc G, Hassapidou M, Spiroski I, et al. WHO European Childhood Obesity Surveillance Initiative: body mass index and level of overweight among 6-9-year-old children from school year 2007/2008 to school year 2009/2010. BMC public health. 2014; 14:806.

24. Ogden CL, Carroll MD, Fryar CD, Flegal KM. Prevalence of Obesity Among Adults and Youth: United States, 2011-2014. NCHS data brief. 2015(219):1-8.

25. Wijnhoven TM, van Raaij JM, Spinelli A, Rito AI, Hovengen R, Kunesova M, et al. WHO European Childhood Obesity Surveillance Initiative 2008: weight, height and body mass index in 6-9-year-old children. Pediatric obesity. 2013; 8(2):79-97.

26. Aarestrup J, Bjerregaard LG, Gamborg M, Angquist L, Tjonneland A, Overvad K, et al. Tracking of body mass index from 7 to 69 years of age. International journal of obesity (2005). 2016

27. Evensen E, Wilsgaard T, Furberg AS, Skeie G. Tracking of overweight and obesity from early childhood to adolescence in a population-based cohort - the Tromso Study, Fit Futures. BMC pediatrics. 2016; 16:64.

28. Yan Y, Hou D, Liang Y, Zhao X, Hu Y, Liu J, et al. Tracking Body Mass Index From Childhood to Adulthood for Subclinical Cardiovascular Diseases at Adulthood. J Am Coll Cardiol. 2016; 67(8):1006-7.

29. Juonala M, Magnussen CG, Berenson GS, Venn A, Burns TL, Sabin MA, et al. Childhood adiposity, adult adiposity, and cardiovascular risk factors. The New England journal of medicine. 2011; 365(20):1876-85.

30. Juonala M, Viikari JS, Ronnemaa T, Marniemi J, Jula A, Loo BM, et al. Associations of dyslipidemias from childhood to adulthood with carotid intima-media thickness, elasticity, and brachial flow-mediated dilatation in adulthood: the Cardiovascular Risk in Young Finns Study. Arteriosclerosis, thrombosis, and vascular biology. 2008; 28(5):1012-7.

31. Kelly RK, Thomson R, Smith KJ, Dwyer T, Venn A, Magnussen CG. Factors Affecting Tracking of Blood Pressure from Childhood to Adulthood: The Childhood Determinants of Adult Health Study. The Journal of pediatrics. 2015; 167(6):1422-8.e2.

32. Currie C. Social determinants of health and well-being among young people. World Health Organization Regional Office for Europe Copenhagen, Denmark; 2012.

33. Marques A, de Matos MG. Trends in prevalence of overweight and obesity: are Portuguese adolescents still increasing weight? International journal of public health. 2016; 61(1):49-56.

34. D'Adamo E, Caprio S. Type 2 diabetes in youth: epidemiology and pathophysiology. Diabetes care. 2011; 34(Supplement 2):S161-S65.

35. Dabelea D, Mayer-Davis EJ, Saydah S, Imperatore G, Linder B, Divers J, et al. Prevalence of type 1 and type 2 diabetes among children and adolescents from 2001 to 2009. Jama. 2014; 311(17):1778-86.

36. Duncan MJ, Vale S, Santos MP, Ribeiro JC, Mota J. The Association between Cardiovascular Disease Risk and Parental Educational Level in Portuguese Children. International Journal of Environmental Research and Public Health. 2012; 9(12):4311-20.

37. May AL, Kuklina EV, Yoon PW. Prevalence of cardiovascular disease risk factors among US adolescents, 1999-2008. Pediatrics. 2012; 129(6):1035-41.

38. Antunes A, Moreira P. [Prevalence of overweight and obesity in Portuguese children and adolescents]. Acta medica portuguesa. 2011; 24(2):279-84.

39. Tanner JM, Whitehouse RH. Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. Archives of disease in childhood. 1976; 51(3):170-9.

40. Traggiai C, Stanhope R. Disorders of pubertal development. Best practice & research Clinical obstetrics & gynaecology. 2003; 17(1):41-56.

41. Morris NM, Udry JR. Validation of a self-administered instrument to assess stage of adolescent development. Journal of youth and adolescence. 1980; 9(3):271-80.

42. Stang J, Story M. Guidelines for adolescent nutrition services. Minneapolis, MN: Center for Leadership. Education and Training in Maternal and Child Nutrition, Division of Epidemiology and Community Health, School of Public Health, University of Minnesota. 2005; 9:101-08.

43. Insel P, Ross D, McMahon K, Bernstein M. Nutrition. 5th ed.: Jones & Bartlett Publishers; 2014.

44. Brown JE. Nutrition through the life cycle. 5th ed.: Cengage Learning; 2014.

45. Flynn MA, McNeil DA, Maloff B, Mutasingwa D, Wu M, Ford C, et al. Reducing obesity and related chronic disease risk in children and youth: a synthesis of evidence with 'best practice' recommendations. Obesity reviews : an official journal of the International Association for the Study of Obesity. 2006; 7 Suppl 1:7-66.

46. Rogol AD, Roemmich JN, Clark PA. Growth at puberty. The Journal of adolescent health : official publication of the Society for Adolescent Medicine. 2002; 31(6 Suppl):192-200.

47. Spear BA. Adolescent growth and development. Journal of the American Dietetic Association. 2002; 102(3 Suppl):S23-9.

48. Gidding SS, Dennison BA, Birch LL, Daniels SR, Gillman MW, Lichtenstein AH, et al. Dietary recommendations for children and adolescents: a guide for practitioners. Pediatrics. 2006; 117(2):544-59.

49. Committee on Physical Activity and Physical Education in the School Environment, Food Nutrition Board, Institute of Medicine. In: Kohl HW, III, Cook HD, editores. Educating the Student Body: Taking Physical Activity and Physical Education to School. Washington (DC): National Academies Press (US); 2013.

50. Ford ES, Ajani UA, Mokdad AH. The metabolic syndrome and concentrations of C-reactive protein among U.S. youth. Diabetes care. 2005; 28(4):878-81.

51. Jarvisalo MJ, Harmoinen A, Hakanen M, Paakkunainen U, Viikari J, Hartiala J, et al. Elevated serum C-reactive protein levels and early arterial changes in healthy children. Arteriosclerosis, thrombosis, and vascular biology. 2002; 22(8):1323-8.

52. Warburton DE, Nicol CW, Bredin SS. Health benefits of physical activity: the evidence. CMAJ : Canadian Medical Association journal = journal de l'Association medicale canadienne. 2006; 174(6):801-9.

53. Currie C, Molcho M, Boyce W, Holstein B, Torsheim T, Richter M. Researching health inequalities in adolescents: the development of the Health Behaviour in School-Aged Children (HBSC) family affluence scale. Social science & medicine (1982). 2008; 66(6):1429-36.

54. Pietras SA, Goodman E. Socioeconomic status gradients in inflammation in adolescence. Psychosomatic medicine. 2013; 75(5):442-8.

55. Chiang JJ, Bower JE, Almeida DM, Irwin MR, Seeman TE, Fuligni AJ. Socioeconomic status, daily affective and social experiences, and inflammation during adolescence. Psychosomatic medicine. 2015; 77(3):256-66.

56. Brummett BH, Babyak MA, Singh A, Jiang R, Williams RB, Harris KM, et al. Socioeconomic indices as independent correlates of C-reactive protein in the National Longitudinal Study of Adolescent Health. Psychosomatic medicine. 2013; 75(9):882-93.

57. Otten JJ, Hellwig JP, Meyers LD. Dietary reference intakes: the essential guide to nutrient requirements. National Academies Press; 2006.

58. Trumbo P, Schlicker S, Yates AA, Poos M. Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and amino acids. Journal of the American Dietetic Association. 2002; 102(11):1621-30.

59. Institute of Medicine, Food and Nutrition Board. Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein and Amino Acids.: National Academies Press; 2005.

60. Institute of Medicine, Food and Nutrition Board. Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride. In. Washington (DC): National Academies Press (US)
National Academy of Sciences.; 1997.

61. Institute of Medicine, Food and Nutrition Board. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. In. Washington (DC): National Academies Press (US)
Copyright 2001 by the National Academy of Sciences. All rights reserved.; 2001.

62. Institute of Medicine, Food and Nutrition Board. Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids. In: Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids. Washington (DC): National Academies Press (US)
Copyright 2000 by the National Academy of Sciences. All rights reserved.; 2000.

63. Institute of Medicine, Food and Nutrition Board. Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline. In. Washington (DC): National Academies Press (US)
National Academy of Sciences.; 1998.

64. Nishida C. Guideline: Sugars intake for adults and children. Geneva: World Health Organization; 2015.

65. Hare-Bruun H, Nielsen BM, Grau K, Oxlund AL, Heitmann BL. Should glycemic index and glycemic load be considered in dietary recommendations? Nutrition reviews. 2008; 66(10):569-90.

66. Buyken AE, Goletzke J, Joslowski G, Felbick A, Cheng G, Herder C, et al. Association between carbohydrate quality and inflammatory markers: systematic review of observational and interventional studies. The American journal of clinical nutrition. 2014; 99(4):813-33.

67. Dahl WJ, Stewart ML. Position of the Academy of Nutrition and Dietetics: Health Implications of Dietary Fiber. Journal of the Academy of Nutrition and Dietetics. 2015; 115(11):1861-70.

68. Vannice G, Rasmussen H. Position of the academy of nutrition and dietetics: dietary fatty acids for healthy adults. Journal of the Academy of Nutrition and Dietetics. 2014; 114(1):136-53.

69. Calder PC, Ahluwalia N, Brouns F, Buetler T, Clement K, Cunningham K, et al. Dietary factors and low-grade inflammation in relation to overweight and obesity. The British journal of nutrition. 2011; 106 Suppl 3:S5-78.

70. Minihane AM, Vinoy S, Russell WR, Baka A, Roche HM, Tuohy KM, et al. Low-grade inflammation, diet composition and health: current research evidence and its translation. The British journal of nutrition. 2015; 114(7):999-1012.

71. Osterholm EA, Georgieff MK. Chronic Inflammation and Iron Metabolism. The Journal of pediatrics. 2015; 166(6):1351-57.e1.

72. Prasad AS. Zinc is an Antioxidant and Anti-Inflammatory Agent: Its Role in Human Health. Frontiers in Nutrition. 2014; 1:14.

73. Trumbo P, Yates AA, Schlicker S, Poos M. Dietary reference intakes: vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. Journal of the American Dietetic Association. 2001; 101(3):294-301.

74. Reifen R. Vitamin A as an anti-inflammatory agent. The Proceedings of the Nutrition Society. 2002; 61(3):397-400.

75. Rodrigues SS, Franchini B, Graca P, de Almeida MD. A new food guide for the Portuguese population: development and technical considerations. Journal of nutrition education and behavior. 2006; 38(3):189-95.

76. Weaver CM. Nutrition and Bone Health. Oral diseases. 2016

77. Boeing H, Bechthold A, Bub A, Ellinger S, Haller D, Kroke A, et al. Critical review: vegetables and fruit in the prevention of chronic diseases. European journal of nutrition. 2012; 51(6):637-63.

78. World Health Organization. Diet, nutrition and the prevention of chronic diseases. 2003/05/29 ed. World Health Organization technical report series. 2003. Technical Report
Switzerland
World Health Organ Tech Rep Ser. 2003;916:i-viii, 1-149, backcover.

79. Lock K, Pomerleau J, Causer L, Altmann DR, McKee M. The global burden of disease attributable to low consumption of fruit and vegetables: implications for the global strategy on diet. Bulletin of the World Health Organization. 2005; 83(2):100-8.

80. Ashfield-Watt PA, Welch AA, Day NE, Bingham SA. Is 'five-a-day' an effective way of increasing fruit and vegetable intakes? Public health nutrition. 2004; 7(2):257-61.

81. World Health Organization. Food based dietary guidelines in the WHO European Region. Organization WH. Copenhagen, Denmark; 2003.

82. U.S. Department of Health and Human Services, and U.S. Department of Agriculture. 2015–2020 Dietary Guidelines for Americans. 8th ed.: US Government Printing Office Washington, DC; 2015. Disponível em: http://health.gov/dietaryguidelines/2015/guidelines/.

83. Lambert J, Agostoni C, Elmadfa I, Hulshof K, Krause E, Livingstone B, et al. Dietary intake and nutritional status of children and adolescents in Europe. The British journal of nutrition. 2004; 92 Suppl 2:S147-211.

84. Araujo J, Severo M, Lopes C, Ramos E. Food sources of nutrients among 13-year-old Portuguese adolescents. Public health nutrition. 2011; 14(11):1970-8.

85. Cruz JA. Dietary habits and nutritional status in adolescents over Europe--Southern Europe. European journal of clinical nutrition. 2000; 54 Suppl 1:S29-35.

86. Araújo J, Teixeira J, Gaio AR, Lopes C, Ramos E. Dietary patterns among 13-y-old Portuguese adolescents. Nutrition (Burbank, Los Angeles County, Calif). 2015; 31(1):148-54.

87. Hibell B, Guttormsson U, Ahlström S, Balakireva O, Bjarnason T, Kokkevi A, et al. The 2011 ESPAD Report. Substance Use among Students in. 2012; 36:123-34.

88. Looze M, Raaijmakers Q, Bogt TT, Bendtsen P, Farhat T, Ferreira M, et al. Decreases in adolescent weekly alcohol use in Europe and North America: evidence from 28 countries from 2002 to 2010. European journal of public health. 2015; 25 Suppl 2:69-72.

89. Abreu S, Santos R, Moreira C, Vale S, Santos PC, Soares-Miranda L, et al. Association between dairy product intake and abdominal obesity in Azorean adolescents. European journal of clinical nutrition. 2012; 66(7):830-5.

90. Al Ani MF, Al Subhi LK, Bose S. Consumption of fruits and vegetables among adolescents: a multi-national comparison of eleven countries in the Eastern Mediterranean Region. The British journal of nutrition. 2016; 115(6):1092-9.

91. Lynch C, Kristjansdottir AG, Te Velde SJ, Lien N, Roos E, Thorsdottir I, et al. Fruit and vegetable consumption in a sample of 11-year-old children in ten European countries--the PRO GREENS cross-sectional survey. Public health nutrition. 2014; 17(11):2436-44.

92. Yngve A, Wolf A, Poortvliet E, Elmadfa I, Brug J, Ehrenblad B, et al. Fruit and vegetable intake in a sample of 11-year-old children in 9 European countries: The Pro Children Cross-sectional Survey. Annals of nutrition & metabolism. 2005; 49(4):236-45.

93. Fismen AS, Smith OR, Torsheim T, Rasmussen M, Pedersen Pagh T, Augustine L, et al. Trends in Food Habits and Their Relation to Socioeconomic Status among Nordic Adolescents 2001/2002-2009/2010. PloS one. 2016; 11(2):e0148541.

94. Fraga S, Severo M, Costa D, Lopes C, Ramos E. Clustering behaviours among 13-year-old Portuguese adolescents. Journal of Public Health. 2011; 19(1):21-27.

95. Vereecken C, Pedersen TP, Ojala K, Krolner R, Dzielska A, Ahluwalia N, et al. Fruit and vegetable consumption trends among adolescents from 2002 to 2010 in 33 countries. European journal of public health. 2015; 25 Suppl 2:16-9.

96. Vereecken C, Dupuy M, Rasmussen M, Kelly C, Nansel TR, Al Sabbah H, et al. Breakfast consumption and its socio-demographic and lifestyle correlates in schoolchildren in 41 countries participating in the HBSC study. International journal of public health. 2009; 54 Suppl 2:180-90.

97. Braithwaite I, Stewart AW, Hancox RJ, Beasley R, Murphy R, Mitchell EA, et al. Fast-food consumption and body mass index in children and adolescents: an international cross-sectional study. BMJ Open. 2014; 4(12):e005813.

98. Moreira P, Santos S, Padrão P, Cordeiro T, Bessa M, Valente H, et al. Food Patterns According to Sociodemographics, Physical Activity, Sleeping and Obesity in Portuguese Children. International Journal of Environmental Research and Public Health. 2010; 7(3):1121-38.

99. Ramos E, Costa A, Araujo J, Severo M, Lopes C. Effect of television viewing on food and nutrient intake among adolescents. Nutrition (Burbank, Los Angeles County, Calif). 2013; 29(11-12):1362-7.

100. Calder PC, Ahluwalia N, Albers R, Bosco N, Bourdet-Sicard R, Haller D, et al. A consideration of biomarkers to be used for evaluation of inflammation in human nutritional studies. The British journal of nutrition. 2013; 109 Suppl 1:S1-34.

101. Fernandes JV, Cobucci RN, Jatoba CA, Fernandes TA, de Azevedo JW, de Araujo JM. The role of the mediators of inflammation in cancer development. Pathology oncology research : POR. 2015; 21(3):527-34.

102. Ostan R, Lanzarini C, Pini E, Scurti M, Vianello D, Bertarelli C, et al. Inflammaging and cancer: a challenge for the Mediterranean diet. Nutrients. 2015; 7(4):2589-621.

103. Medzhitov R. Origin and physiological roles of inflammation. Nature. 2008; 454(7203):428-35.

104. Minciullo PL, Catalano A, Mandraffino G, Casciaro M, Crucitti A, Maltese G, et al. Inflammaging and Anti-Inflammaging: The Role of Cytokines in Extreme Longevity. Archivum immunologiae et therapiae experimentalis. 2016; 64(2):111-26.

105. Jenny NS. Inflammation in aging: cause, effect, or both? Discovery medicine. 2012; 13(73):451-60.

106. Galcheva SV, Iotova VM, Yotov YT, Bernasconi S, Street ME. Circulating proinflammatory peptides related to abdominal adiposity and cardiometabolic risk factors in healthy prepubertal children. European journal of endocrinology / European Federation of Endocrine Societies. 2011; 164(4):553-8.

107. Warnberg J, Nova E, Moreno LA, Romeo J, Mesana MI, Ruiz JR, et al. Inflammatory proteins are related to total and abdominal adiposity in a healthy adolescent population: the AVENA Study. The American journal of clinical nutrition. 2006; 84(3):505-12.

108. Carraro JC, Hermsdorff HH, Puchau B, Zulet MA, Milagro FI, Bressan J, et al. Interleukin-6 is a better metabolic biomarker than interleukin-18 in young healthy adults. Journal of physiology and biochemistry. 2015; 71(3):527-35.

109. Ridker PM. From C-Reactive Protein to Interleukin-6 to Interleukin-1: Moving Upstream To Identify Novel Targets for Atheroprotection. Circulation research. 2016; 118(1):145-56.

110. Soeki T, Sata M. Inflammatory Biomarkers and Atherosclerosis. International heart journal. 2016; 57(2):134-9.

111. Gaillard R, Rifas-Shiman SL, Perng W, Oken E, Gillman MW. Maternal inflammation during pregnancy and childhood adiposity. Obesity (Silver Spring, Md). 2016; 24(6):1320-7.

112. de Ferranti SD, Gauvreau K, Ludwig DS, Newburger JW, Rifai N. Inflammation and changes in metabolic syndrome abnormalities in US adolescents: findings from the 1988-1994 and 1999-2000 National Health and Nutrition Examination Surveys. Clinical chemistry. 2006; 52(7):1325-30.

113. Pilz S, Horejsi R, Moller R, Almer G, Scharnagl H, Stojakovic T, et al. Early atherosclerosis in obese juveniles is associated with low serum levels of adiponectin. The Journal of clinical endocrinology and metabolism. 2005; 90(8):4792-6.

114. Silva LR, Stefanello JM, Pizzi J, Timossi LS, Leite N. Aterosclerose subclínica e marcadores inflamatórios em crianças e adolescentes obesos e não obesos. Revista Brasileira de Epidemiologia. 2012; 15:804-06.

115. Vlaicu SI, Tatomir A, Boodhoo D, Vesa S, Mircea PA, Rus H. The role of complement system in adipose tissue-related inflammation. Immunologic research. 2016; 64(3):653-64.

116. Onat A, Can G, Rezvani R, Cianflone K. Complement C3 and cleavage products in cardiometabolic risk. Clinica chimica acta; international journal of clinical chemistry. 2011; 412(13-14):1171-9.

117. Nilsson B, Hamad OA, Ahlstrom H, Kullberg J, Johansson L, Lindhagen L, et al. C3 and C4 are strongly related to adipose tissue variables and cardiovascular risk factors. European journal of clinical investigation. 2014; 44(6):587-96.

118. Oliveira-Santos J, Santos R, Moreira C, Abreu S, Lopes L, Agostinis C, et al. Ability of Measures of Adiposity in Identifying Adverse Levels of Inflammatory and Metabolic Markers in Adolescents. Childhood obesity (Print). 2016; 12(2):135-43.

119. Engstrom G, Hedblad B, Janzon L, Lindgarde F. Complement C3 and C4 in plasma and incidence of myocardial infarction and stroke: a population-based cohort study. European journal of cardiovascular prevention and rehabilitation : official journal of the European Society of Cardiology, Working Groups on Epidemiology & Prevention and Cardiac Rehabilitation and Exercise Physiology. 2007; 14(3):392-7.

120. Palikhe A, Sinisalo J, Seppanen M, Haario H, Meri S, Valtonen V, et al. Serum complement C3/C4 ratio, a novel marker for recurrent cardiovascular events. The American journal of cardiology. 2007; 99(7):890-5.

121. Nestvold TK, Nielsen EW, Ludviksen JK, Fure H, Landsem A, Lappegard KT. Lifestyle changes followed by bariatric surgery lower inflammatory markers and the cardiovascular risk factors C3 and C4. Metabolic syndrome and related disorders. 2015; 13(1):29-35.

122. Martos-Moreno GA, Barrios V, Chowen JA, Argente J. Adipokines in childhood obesity. Vitamins and hormones. 2013; 91:107-42.

123. Yudkin JS, Kumari M, Humphries SE, Mohamed-Ali V. Inflammation, obesity, stress and coronary heart disease: is interleukin-6 the link? Atherosclerosis. 2000; 148(2):209-14.

124. Fantuzzi G. Adipose tissue, adipokines, and inflammation. The Journal of allergy and clinical immunology. 2005; 115(5):911-9; quiz 20.

125. Maggio M, Guralnik JM, Longo DL, Ferrucci L. Interleukin-6 in aging and chronic disease: a magnificent pathway. The journals of gerontology Series A, Biological sciences and medical sciences. 2006; 61(6):575-84.

126. Perez CM, Ortiz AP, Fuentes-Mattei E, Velazquez-Torres G, Santiago D, Giovannetti K, et al. High prevalence of cardiometabolic risk factors in Hispanic adolescents: correlations with adipocytokines and markers of inflammation. Journal of immigrant and minority health / Center for Minority Public Health. 2014; 16(5):865-73.

127. Bugge A, El-Naaman B, McMurray RG, Froberg K, Nielsen CH, Muller K, et al. Inflammatory markers and clustered cardiovascular disease risk factors in Danish adolescents. Hormone research in paediatrics. 2012; 78(5-6):288-96.

128. Gonzalez-Jimenez E, Schmidt-Riovalle J, Sinausia L, Carmen Valenza M, Perona JS. Predictive value of ceruloplasmin for metabolic syndrome in adolescents. BioFactors (Oxford, England). 2016; 42(2):163-70.

129. Eder K, Baffy N, Falus A, Fulop AK. The major inflammatory mediator interleukin-6 and obesity. Inflammation research : official journal of the European Histamine Research Society [et al]. 2009; 58(11):727-36.

130. Mauer J, Chaurasia B, Goldau J, Vogt MC, Ruud J, Nguyen KD, et al. Signaling by IL-6 promotes alternative activation of macrophages to limit endotoxemia and obesity-associated resistance to insulin. Nature immunology. 2014; 15(5):423-30.

131. Sindhu S, Thomas R, Shihab P, Sriraman D, Behbehani K, Ahmad R. Obesity Is a Positive Modulator of IL-6R and IL-6 Expression in the Subcutaneous Adipose Tissue: Significance for Metabolic Inflammation. PloS one. 2015; 10(7):e0133494.

132. Caminiti C, Armeno M, Mazza CS. Waist-to-height ratio as a marker of low-grade inflammation in obese children and adolescents. Journal of pediatric endocrinology & metabolism : JPEM. 2016; 29(5):543-51.

133. Gonzalez M, del Mar Bibiloni M, Pons A, Llompart I, Tur JA. Inflammatory markers and metabolic syndrome among adolescents. European journal of clinical nutrition. 2012; 66(10):1141-5.

134. Caballero AE, Bousquet-Santos K, Robles-Osorio L, Montagnani V, Soodini G, Porramatikul S, et al. Overweight Latino children and adolescents have marked endothelial dysfunction and subclinical vascular inflammation in association with excess body fat and insulin resistance. Diabetes care. 2008; 31(3):576-82.

135. Pardo Morales RV, Zuniga Torres MG, Martinez Carrillo BE, Gomez Martinez S, Marcos A, Valdes Ramos R. Inflammatory mediators and immune response in Mexican adolescents. Nutricion hospitalaria. 2011; 26(5):1115-9.

136. Tam CS, Garnett SP, Cowell CT, Heilbronn LK, Lee JW, Wong M, et al. IL-6, IL-8 and IL-10 levels in healthy weight and overweight children. Hormone research in paediatrics. 2010; 73(2):128-34.

137. Nieto-Vazquez I, Fernandez-Veledo S, Kramer DK, Vila-Bedmar R, Garcia-Guerra L, Lorenzo M. Insulin resistance associated to obesity: the link TNF-alpha. Archives of physiology and biochemistry. 2008; 114(3):183-94.

138. Balagopal PB, de Ferranti SD, Cook S, Daniels SR, Gidding SS, Hayman LL, et al. Nontraditional risk factors and biomarkers for cardiovascular disease: mechanistic, research, and clinical considerations for youth: a scientific statement from the American Heart Association. Circulation. 2011; 123(23):2749-69.

139. Dandona P, Aljada A, Bandyopadhyay A. Inflammation: the link between insulin resistance, obesity and diabetes. Trends in immunology. 2004; 25(1):4-7.

140. Zou C, Shao J. Role of adipocytokines in obesity-associated insulin resistance. The Journal of nutritional biochemistry. 2008; 19(5):277-86.

141. Tzanavari T, Giannogonas P, Karalis KP. TNF-alpha and obesity. Current directions in autoimmunity. 2010; 11:145-56.

142. Mendelson M, Michallet AS, Monneret D, Perrin C, Esteve F, Lombard PR, et al. Impact of exercise training without caloric restriction on inflammation, insulin resistance and visceral fat mass in obese adolescents. Pediatric obesity. 2015; 10(4):311-9.

143. Cohen JI, Maayan L, Convit A. Preliminary evidence for obesity-associated insulin resistance in adolescents without elevations of inflammatory cytokines. Diabetology & metabolic syndrome. 2012; 4(1):26.

144. Rigamonti AE, Agosti F, De Col A, Silvestri G, Marazzi N, Bini S, et al. Severely obese adolescents and adults exhibit a different association of circulating levels of adipokines and leukocyte expression of the related receptors with insulin resistance. International journal of endocrinology. 2013; 2013:565967.

145. Rubin DA, McMurray RG, Hackney AC, Harrell JS. Relationship between cardiovascular risk factors and adipokines in adolescents. Hormone research in paediatrics. 2011; 76(2):123-9.

146. Moon YS, Kim DH, Song DK. Serum tumor necrosis factor-alpha levels and components of the metabolic syndrome in obese adolescents. Metabolism: clinical and experimental. 2004; 53(7):863-7.

147. Steene-Johannessen J, Kolle E, Reseland JE, Anderssen SA, Andersen LB. Waist circumference is related to low-grade inflammation in youth. International journal of pediatric obesity : IJPO : an official journal of the International Association for the Study of Obesity. 2010; 5(4):313-9.

148. Pires A, Martins P, Pereira AM, Marinho J, Vaz Silva P, Marques M, et al. Pro-inflammatory triggers in childhood obesity: correlation between leptin, adiponectin and high-sensitivity C-reactive protein in a group of obese Portuguese children. Revista portuguesa de cardiologia : orgao oficial da Sociedade Portuguesa de Cardiologia = Portuguese journal of cardiology : an official journal of the Portuguese Society of Cardiology. 2014; 33(11):691-7.

149. El-Wakkad A, Hassan Nel M, Sibaii H, El-Zayat SR. Proinflammatory, anti-inflammatory cytokines and adiponkines in students with central obesity. Cytokine. 2013; 61(2):682-7.

150. Chang CJ, Jian DY, Lin MW, Zhao JZ, Ho LT, Juan CC. Evidence in obese children: contribution of hyperlipidemia, obesity-inflammation, and insulin sensitivity. PloS one. 2015; 10(5):e0125935.

151. Nascimento H, Costa E, Rocha S, Lucena C, Rocha-Pereira P, Rego C, et al. Adiponectin and markers of metabolic syndrome in obese children and adolescents: impact of 8-mo regular physical exercise program. Pediatric research. 2014; 76(2):159-65.

152. Klunder-Klunder M, Flores-Huerta S, Garcia-Macedo R, Peralta-Romero J, Cruz M. Adiponectin in eutrophic and obese children as a biomarker to predict metabolic syndrome and each of its components. BMC public health. 2013; 13:88.

153. Weiss R, Dufour S, Groszmann A, Petersen K, Dziura J, Taksali SE, et al. Low adiponectin levels in adolescent obesity: a marker of increased intramyocellular lipid accumulation. The Journal of clinical endocrinology and metabolism. 2003; 88(5):2014-8.

154. Aguilar MJ, Gonzalez-Jimenez E, Antelo A, Perona JS. Insulin resistance and inflammation markers: correlations in obese adolescents. Journal of clinical nursing. 2013; 22(13-14):2002-10.

155. Winer JC, Zern TL, Taksali SE, Dziura J, Cali AM, Wollschlager M, et al. Adiponectin in childhood and adolescent obesity and its association with inflammatory markers and components of the metabolic syndrome. The Journal of clinical endocrinology and metabolism. 2006; 91(11):4415-23.

156. Beauloye V, Zech F, Tran HT, Clapuyt P, Maes M, Brichard SM. Determinants of early atherosclerosis in obese children and adolescents. The Journal of clinical endocrinology and metabolism. 2007; 92(8):3025-32.

157. Adabimohazab R, Garfinkel A, Milam EC, Frosch O, Mangone A, Convit A. Does Inflammation Mediate the Association Between Obesity and Insulin Resistance? Inflammation. 2016; 39(3):994-1003.

158. Artero EG, Espana-Romero V, Jimenez-Pavon D, Martinez-Gomez D, Warnberg J, Gomez-Martinez S, et al. Muscular fitness, fatness and inflammatory biomarkers in adolescents. Pediatric obesity. 2014; 9(5):391-400.

159. Going SB, Lohman TG, Cussler EC, Williams DP, Morrison JA, Horn PS. Percent body fat and chronic disease risk factors in U.S. children and youth. American journal of preventive medicine. 2011; 41(4 Suppl 2):S77-86.

160. DeLoach S, Keith SW, Gidding SS, Falkner B. Obesity associated inflammation in African American adolescents and adults. The American journal of the medical sciences. 2014; 347(5):357-63.

161. Cook DG, Mendall MA, Whincup PH, Carey IM, Ballam L, Morris JE, et al. C-reactive protein concentration in children: relationship to adiposity and other cardiovascular risk factors. Atherosclerosis. 2000; 149(1):139-50.

162. Martinez-Gomez D, Eisenmann JC, Warnberg J, Gomez-Martinez S, Veses A, Veiga OL, et al. Associations of physical activity, cardiorespiratory fitness and fatness with low-grade inflammation in adolescents: the AFINOS Study. International journal of obesity (2005). 2010; 34(10):1501-7.

163. Ruiz JR, Ortega FB, Warnberg J, Sjostrom M. Associations of low-grade inflammation with physical activity, fitness and fatness in prepubertal children;

the European Youth Heart Study. International journal of obesity (2005). 2007; 31(10):1545-51.

164. Shatat IF, Freeman KD, Vuguin PM, Dimartino-Nardi JR, Flynn JT. Relationship between adiponectin and ambulatory blood pressure in obese adolescents. Pediatric research. 2009; 65(6):691-5.

165. Andersen LB, Muller K, Eiberg S, Froberg K, Andersen JF, Bugge A, et al. Cytokines and clustered cardiovascular risk factors in children. Metabolism: clinical and experimental. 2010; 59(4):561-6.

166. Aeberli I, Molinari L, Spinas G, Lehmann R, l'Allemand D, Zimmermann MB. Dietary intakes of fat and antioxidant vitamins are predictors of subclinical inflammation in overweight Swiss children. The American journal of clinical nutrition. 2006; 84(4):748-55.

167. Bugge A, El-Naaman B, R GM, Froberg K, Nielsen CH, Muller K, et al. Sex differences in the association between level of childhood interleukin-6 and insulin resistance in adolescence. Experimental diabetes research. 2012; 2012:859186.

168. Rubin DA, McMurray RG, Harrell JS, Hackney AC, Thorpe DE, Haqq AM. The association between insulin resistance and cytokines in adolescents: the role of weight status and exercise. Metabolism: clinical and experimental. 2008; 57(5):683-90.

169. Zabaleta J, Velasco-Gonzalez C, Estrada J, Ravussin E, Pelligrino N, Mohler MC, et al. Inverse correlation of serum inflammatory markers with metabolic parameters in healthy, Black and White prepubertal youth. International journal of obesity (2005). 2014; 38(4):563-8.

170. Alikasifoglu A, Gonc N, Ozon ZA, Sen Y, Kandemir N. The relationship between serum adiponectin, tumor necrosis factor-alpha, leptin levels and insulin sensitivity in childhood and adolescent obesity: adiponectin is a marker of metabolic syndrome. Journal of clinical research in pediatric endocrinology. 2009; 1(5):233-9.

171. Gregor MF, Hotamisligil GS. Inflammatory mechanisms in obesity. Annual review of immunology. 2011; 29:415-45.

172. Tremblay MS, Colley RC, Saunders TJ, Healy GN, Owen N. Physiological and health implications of a sedentary lifestyle. Applied physiology, nutrition, and metabolism = Physiologie appliquee, nutrition et metabolisme. 2010; 35(6):725-40.

173. Rom O, Avezov K, Aizenbud D, Reznick AZ. Cigarette smoking and inflammation revisited. Respiratory physiology & neurobiology. 2013; 187(1):5-10.

174. Nimmo MA, Leggate M, Viana JL, King JA. The effect of physical activity on mediators of inflammation. Diabetes, obesity & metabolism. 2013; 15 Suppl 3:51-60.

175. Fraga S, Marques-Vidal P, Vollenweider P, Waeber G, Guessous I, Paccaud F, et al. Association of socioeconomic status with inflammatory markers: a two cohort comparison. Preventive medicine. 2015; 71:12-9.

176. Koster A, Bosma H, Penninx BW, Newman AB, Harris TB, van Eijk JT, et al. Association of inflammatory markers with socioeconomic status. The journals of gerontology Series A, Biological sciences and medical sciences. 2006; 61(3):284-90.

177. Freeman JA, Bauldry S, Volpe VV, Shanahan MJ, Shanahan L. Sex Differences in Associations Between Subjective Social Status and C-Reactive Protein in Young Adults. Psychosomatic medicine. 2016; 78(5):542-51.

178. Schmeer KK, Yoon A. Socioeconomic status inequalities in low-grade inflammation during childhood. Archives of disease in childhood. 2016

179. Tam CS, Clement K, Baur LA, Tordjman J. Obesity and low-grade inflammation: a paediatric perspective. Obesity reviews : an official journal of the International Association for the Study of Obesity. 2010; 11(2):118-26.

180. Ford ES. Body mass index, diabetes, and C-reactive protein among U.S. adults. Diabetes care. 1999; 22(12):1971-7.

181. Ford ES, Giles WH, Myers GL, Rifai N, Ridker PM, Mannino DM. C-reactive protein concentration distribution among US children and young adults: findings from the National Health and Nutrition Examination Survey, 1999-2000. Clinical chemistry. 2003; 49(8):1353-7.

182. Choi J, Joseph L, Pilote L. Obesity and C-reactive protein in various populations: a systematic review and meta-analysis. Obesity reviews : an official journal of the International Association for the Study of Obesity. 2013; 14(3):232-44.

183. Shanahan L, Bauldry S, Freeman J, Bondy CL. Self-rated health and C-reactive protein in young adults. Brain, behavior, and immunity. 2014; 36:139-46.

184. Ahonen T, Vanhala M, Kautiainen H, Kumpusalo E, Saltevo J. Sex differences in the association of adiponectin and low-grade inflammation with changes in the body mass index from youth to middle age. Gender medicine. 2012; 9(1):1-8.

185. Pirkola J, Vaarasmaki M, Ala-Korpela M, Bloigu A, Canoy D, Hartikainen AL, et al. Low-grade, systemic inflammation in adolescents: association with early-life factors, gender, and lifestyle. Am J Epidemiol. 2010; 171(1):72-82.

186. Meas T, Deghmoun S, Armoogum P, Alberti C, Levy-Marchal C. Consequences of Being Born Small for Gestational Age on Body Composition: An 8-Year Follow-Up Study. The Journal of clinical endocrinology and metabolism. 2008; 93(10):3804-09.

187. Dosch NC, Guslits EF, Weber MB, Murray SE, Ha B, Coe CL, et al. Maternal Obesity Affects Inflammatory and Iron Indices in Umbilical Cord Blood. The Journal of pediatrics. 2016; 172:20-8.

188. Rubin DA, Hackney AC. Inflammatory cytokines and metabolic risk factors during growth and maturation: influence of physical activity. Medicine and sport science. 2010; 55:43-55.

189. Martinez-Gomez D, Gomez-Martinez S, Ruiz JR, Diaz LE, Ortega FB, Widhalm K, et al. Objectively-measured and self-reported physical activity and fitness in relation to inflammatory markers in European adolescents: the HELENA Study. Atherosclerosis. 2012; 221(1):260-7.

190. Platat C, Wagner A, Klumpp T, Schweitzer B, Simon C. Relationships of physical activity with metabolic syndrome features and low-grade inflammation in adolescents. Diabetologia. 2006; 49(9):2078-85.

191. Pahkala K, Laitinen TT, Heinonen OJ, Viikari JS, Ronnemaa T, Niinikoski H, et al. Association of fitness with vascular intima-media thickness and elasticity in adolescence. Pediatrics. 2013; 132(1):e77-84.

192. Ruiz JR, Ortega FB, Warnberg J, Moreno LA, Carrero JJ, Gonzalez-Gross M, et al. Inflammatory proteins and muscle strength in adolescents: the Avena study. Archives of pediatrics & adolescent medicine. 2008; 162(5):462-8.

193. Allison MA, Jensky NE, Marshall SJ, Bertoni AG, Cushman M. Sedentary behavior and adiposity-associated inflammation: the Multi-Ethnic Study of Atherosclerosis. American journal of preventive medicine. 2012; 42(1):8-13.

194. Yates T, Khunti K, Wilmot EG, Brady E, Webb D, Srinivasan B, et al. Self-reported sitting time and markers of inflammation, insulin resistance, and adiposity. American journal of preventive medicine. 2012; 42(1):1-7.

195. Henson J, Yates T, Edwardson CL, Khunti K, Talbot D, Gray LJ, et al. Sedentary Time and Markers of Chronic Low-Grade Inflammation in a High Risk Population. PloS one. 2013; 8(10):e78350.

196. Martinez-Gomez D, Eisenmann JC, Healy GN, Gomez-Martinez S, Diaz LE, Dunstan DW, et al. Sedentary behaviors and emerging cardiometabolic biomarkers in adolescents. The Journal of pediatrics. 2012; 160(1):104-10.e2.

197. Hardy LL, Denney-Wilson E, Thrift AP, Okely AD, Baur LA. Screen time and metabolic risk factors among adolescents. Archives of pediatrics & adolescent medicine. 2010; 164(7):643-9.

198. Lee H, Lee IS, Choue R. Obesity, inflammation and diet. Pediatric gastroenterology, hepatology & nutrition. 2013; 16(3):143-52.

199. Joffe YT, Collins M, Goedecke JH. The relationship between dietary fatty acids and inflammatory genes on the obese phenotype and serum lipids. Nutrients. 2013; 5(5):1672-705.

200. Santos S, Oliveira A, Lopes C. Systematic review of saturated fatty acids on inflammation and circulating levels of adipokines. Nutrition research (New York, NY). 2013; 33(9):687-95.

201. Arya S, Isharwal S, Misra A, Pandey RM, Rastogi K, Vikram NK, et al. C-reactive protein and dietary nutrients in urban Asian Indian adolescents and young adults. Nutrition (Burbank, Los Angeles County, Calif). 2006; 22(9):865-71.

202. Wang H, Steffen LM, Vessby B, Basu S, Steinberger J, Moran A, et al. Obesity modifies the relations between serum markers of dairy fats and inflammation and oxidative stress among adolescents. Obesity (Silver Spring, Md). 2011; 19(12):2404-10.

203. Gonzalez-Gil EM, Santabarbara J, Siani A, Ahrens W, Sioen I, Eiben G, et al. Whole-blood fatty acids and inflammation in European children: the IDEFICS Study. European journal of clinical nutrition. 2016; 70(7):819-23.

204. Klein-Platat C, Drai J, Oujaa M, Schlienger JL, Simon C. Plasma fatty acid composition is associated with the metabolic syndrome and low-grade inflammation in overweight adolescents. The American journal of clinical nutrition. 2005; 82(6):1178-84.

205. Calder PC. Marine omega-3 fatty acids and inflammatory processes: Effects, mechanisms and clinical relevance. Biochimica et biophysica acta. 2015; 1851(4):469-84.

206. Burdge GC, Calder PC. Dietary alpha-linolenic acid and health-related outcomes: a metabolic perspective. Nutrition research reviews. 2006; 19(1):26-52.

207. Kuo SM. The interplay between fiber and the intestinal microbiome in the inflammatory response. Advances in nutrition (Bethesda, Md). 2013; 4(1):16-28.

208. Bervoets L, Van Hoorenbeeck K, Kortleven I, Van Noten C, Hens N, Vael C, et al. Differences in gut microbiota composition between obese and lean children: a cross-sectional study. Gut Pathogens. 2013; 5:10-10.

209. Ignacio A, Fernandes MR, Rodrigues VA, Groppo FC, Cardoso AL, Avila-Campos MJ, et al. Correlation between body mass index and faecal microbiota from children. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2016; 22(3):258.e1-8.

210. Iannuzzi A, Licenziati MR, Vacca M, De Marco D, Cinquegrana G, Laccetti M, et al. Comparison of two diets of varying glycemic index on carotid subclinical atherosclerosis in obese children. Heart and vessels. 2009; 24(6):419-24.

211. Rouhani MH, Kelishadi R, Hashemipour M, Esmaillzadeh A, Surkan PJ, Keshavarz A, et al. The Impact of a Low Glycemic Index Diet on Inflammatory Markers and Serum Adiponectin Concentration in Adolescent Overweight and Obese Girls: A Randomized Clinical Trial. Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme. 2016; 48(4):251-6.

212. Goletzke J, Buyken AE, Joslowski G, Bolzenius K, Remer T, Carstensen M, et al. Increased intake of carbohydrates from sources with a higher glycemic index and lower consumption of whole grains during puberty are prospectively associated with higher IL-6 concentrations in younger adulthood among healthy individuals. The Journal of nutrition. 2014; 144(10):1586-93.

213. Parikh S, Pollock NK, Bhagatwala J, Guo DH, Gutin B, Zhu H, et al. Adolescent fiber consumption is associated with visceral fat and inflammatory markers. The Journal of clinical endocrinology and metabolism. 2012; 97(8):E1451-7.

214. Holt EM, Steffen LM, Moran A, Basu S, Steinberger J, Ross JA, et al. Fruit and vegetable consumption and its relation to markers of inflammation and oxidative stress in adolescents. Journal of the American Dietetic Association. 2009; 109(3):414-21.

215. Garcia OP, Ronquillo D, del Carmen Caamano M, Martinez G, Camacho M, Lopez V, et al. Zinc, iron and vitamins A, C and e are associated with obesity,

inflammation, lipid profile and insulin resistance in Mexican school-aged children. Nutrients. 2013; 5(12):5012-30.

216. Liu RH. Potential synergy of phytochemicals in cancer prevention: mechanism of action. The Journal of nutrition. 2004; 134(12 Suppl):3479s-85s.

217. Rosa FT, Zulet MA, Marchini JS, Martinez JA. Bioactive compounds with effects on inflammation markers in humans. International journal of food sciences and nutrition. 2012; 63(6):749-65.

218. Serafini M, Peluso I, Raguzzini A. Flavonoids as anti-inflammatory agents. The Proceedings of the Nutrition Society. 2010; 69(3):273-8.

219. Padayatty SJ, Levine M. Fruit and vegetables: think variety, go ahead, eat! The American journal of clinical nutrition. 2008; 87(1):5-7.

220. Kaulmann A, Bohn T. Carotenoids, inflammation, and oxidative stress--implications of cellular signaling pathways and relation to chronic disease prevention. Nutrition research (New York, NY). 2014; 34(11):907-29.

221. Liu RH. Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. The American journal of clinical nutrition. 2003; 78(3 Suppl):517s-20s.

222. Huang Z, Rose AH, Hoffmann PR. The role of selenium in inflammation and immunity: from molecular mechanisms to therapeutic opportunities. Antioxidants & redox signaling. 2012; 16(7):705-43.

223. Del Mar Bibiloni M, Maffeis C, Llompart I, Pons A, Tur JA. Dietary factors associated with subclinical inflammation among girls. European journal of clinical nutrition. 2013; 67(12):1264-70.

224. Murer SB, Aeberli I, Braegger CP, Gittermann M, Hersberger M, Leonard SW, et al. Antioxidant supplements reduced oxidative stress and stabilized liver function tests but did not reduce inflammation in a randomized controlled trial in obese children and adolescents. The Journal of nutrition. 2014; 144(2):193-201.

225. Tussing-Humphreys LM, Liang H, Nemeth E, Freels S, Braunschweig CA. Excess adiposity, inflammation, and iron-deficiency in female adolescents. Journal of the American Dietetic Association. 2009; 109(2):297-302.

226. Ferrari M, Cuenca-Garcia M, Valtuena J, Moreno LA, Censi L, Gonzalez-Gross M, et al. Inflammation profile in overweight/obese adolescents in Europe: an analysis in relation to iron status. European journal of clinical nutrition. 2015; 69(2):247-55.

227. King DE. Inflammation and elevation of C-reactive protein: does magnesium play a key role? Magnesium research. 2009; 22(2):57-9.

228. Guerrero-Romero F, Bermudez-Pena C, Rodriguez-Moran M. Severe hypomagnesemia and low-grade inflammation in metabolic syndrome. Magnesium research. 2011; 24(2):45-53.

229. Guerrero-Romero F, Rodriguez-Moran M. Low serum magnesium levels and metabolic syndrome. Acta diabetologica. 2002; 39(4):209-13.

230. Song Y, Ridker PM, Manson JE, Cook NR, Buring JE, Liu S. Magnesium intake, C-reactive protein, and the prevalence of metabolic syndrome in middle-aged and older U.S. women. Diabetes care. 2005; 28(6):1438-44.

231. King DE, Mainous AG, 3rd, Geesey ME, Woolson RF. Dietary magnesium and C-reactive protein levels. Journal of the American College of Nutrition. 2005; 24(3):166-71.

232. Lopez-Alarcon M, Perichart-Perera O, Flores-Huerta S, Inda-Icaza P, Rodriguez-Cruz M, Armenta-Alvarez A, et al. Excessive refined carbohydrates and scarce micronutrients intakes increase inflammatory mediators and insulin resistance in prepubertal and pubertal obese children independently of obesity. Mediators of inflammation. 2014; 2014:849031.

233. Rodriguez-Moran M, Guerrero-Romero F. Serum magnesium and C-reactive protein levels. Archives of disease in childhood. 2008; 93(8):676-80.

234. King DE, Mainous AG, 3rd, Geesey ME, Ellis T. Magnesium intake and serum C-reactive protein levels in children. Magnesium research. 2007; 20(1):32-6.

235. Ford ES, Mokdad AH, Liu S. Healthy Eating Index and C-reactive protein concentration: findings from the National Health and Nutrition Examination Survey III, 1988-1994. European journal of clinical nutrition. 2005; 59(2):278-83.

236. Lutsey PL, Jacobs DR, Jr., Kori S, Mayer-Davis E, Shea S, Steffen LM, et al. Whole grain intake and its cross-sectional association with obesity, insulin resistance, inflammation, diabetes and subclinical CVD: The MESA Study. The British journal of nutrition. 2007; 98(2):397-405.

237. Masters RC, Liese AD, Haffner SM, Wagenknecht LE, Hanley AJ. Whole and refined grain intakes are related to inflammatory protein concentrations in human plasma. The Journal of nutrition. 2010; 140(3):587-94.

238. Katcher HI, Legro RS, Kunselman AR, Gillies PJ, Demers LM, Bagshaw DM, et al. The effects of a whole grain-enriched hypocaloric diet on cardiovascular disease risk factors in men and women with metabolic syndrome. The American journal of clinical nutrition. 2008; 87(1):79-90.

239. Brownlee IA, Moore C, Chatfield M, Richardson DP, Ashby P, Kuznesof SA, et al. Markers of cardiovascular risk are not changed by increased whole-grain intake: the WHOLEheart study, a randomised, controlled dietary intervention. The British journal of nutrition. 2010; 104(1):125-34.

240. Tighe P, Duthie G, Vaughan N, Brittenden J, Simpson WG, Duthie S, et al. Effect of increased consumption of whole-grain foods on blood pressure and other cardiovascular risk markers in healthy middle-aged persons: a randomized controlled trial. The American journal of clinical nutrition. 2010; 92(4):733-40.

241. Hajihashemi P, Azadbakht L, Hashemipor M, Kelishadi R, Esmaillzadeh A. Whole-grain intake favorably affects markers of systemic inflammation in obese children: a randomized controlled crossover clinical trial. Molecular nutrition & food research. 2014; 58(6):1301-8.

242. van Bussel BC, Henry RM, Ferreira I, van Greevenbroek MM, van der Kallen CJ, Twisk JW, et al. A healthy diet is associated with less endothelial dysfunction and less low-grade inflammation over a 7-year period in adults at risk of cardiovascular disease. The Journal of nutrition. 2015; 145(3):532-40.

243. Bhupathiraju SN, Tucker KL. Greater variety in fruit and vegetable intake is associated with lower inflammation in Puerto Rican adults. The American journal of clinical nutrition. 2011; 93(1):37-46.

244. Qureshi MM, Singer MR, Moore LL. A cross-sectional study of food group intake and C-reactive protein among children. Nutrition & metabolism. 2009; 6:40.

245. Wannamethee SG, Lowe GD, Rumley A, Bruckdorfer KR, Whincup PH. Associations of vitamin C status, fruit and vegetable intakes, and markers of inflammation and hemostasis. The American journal of clinical nutrition. 2006; 83(3):567-74; quiz 726-7.

246. Root MM, McGinn MC, Nieman DC, Henson DA, Heinz SA, Shanely RA, et al. Combined fruit and vegetable intake is correlated with improved inflammatory and oxidant status from a cross-sectional study in a community setting. Nutrients. 2012; 4(1):29-41.

247. Jiang Y, Wu SH, Shu XO, Xiang YB, Ji BT, Milne GL, et al. Cruciferous vegetable intake is inversely correlated with circulating levels of proinflammatory markers in women. Journal of the Academy of Nutrition and Dietetics. 2014; 114(5):700-8.e2.

248. Hermsdorff HH, Zulet MA, Puchau B, Martinez JA. Fruit and vegetable consumption and proinflammatory gene expression from peripheral blood mononuclear cells in young adults: a translational study. Nutrition & metabolism. 2010; 7:42.

249. Gao X, Bermudez OI, Tucker KL. Plasma C-reactive protein and homocysteine concentrations are related to frequent fruit and vegetable intake in Hispanic and non-Hispanic white elders. The Journal of nutrition. 2004; 134(4):913-8.

250. Esmaillzadeh A, Kimiagar M, Mehrabi Y, Azadbakht L, Hu FB, Willett WC. Fruit and vegetable intakes, C-reactive protein, and the metabolic syndrome. The American journal of clinical nutrition. 2006; 84(6):1489-97.

251. Chun OK, Chung SJ, Claycombe KJ, Song WO. Serum C-reactive protein concentrations are inversely associated with dietary flavonoid intake in U.S. adults. The Journal of nutrition. 2008; 138(4):753-60.

252. Oliveira A, Rodriguez-Artalejo F, Lopes C. The association of fruits, vegetables, antioxidant vitamins and fibre intake with high-sensitivity C-reactive protein: sex and body mass index interactions. European journal of clinical nutrition. 2009; 63(11):1345-52.

253. Yannakoulia M, Yiannakouris N, Melistas L, Fappa E, Vidra N, Kontogianni MD, et al. Dietary factors associated with plasma high molecular weight and total adiponectin levels in apparently healthy women. European journal of endocrinology / European Federation of Endocrine Societies. 2008; 159(4):R5-10.

254. Salas-Salvado J, Garcia-Arellano A, Estruch R, Marquez-Sandoval F, Corella D, Fiol M, et al. Components of the Mediterranean-type food pattern and serum inflammatory markers among patients at high risk for cardiovascular disease. European journal of clinical nutrition. 2008; 62(5):651-9.

255. Ko A, Kim H, Han CJ, Kim JM, Chung HW, Chang N. Association between high sensitivity C-reactive protein and dietary intake in Vietnamese young women. Nutrition research and practice. 2014; 8(4):445-52.

256. Navarro SL, Schwarz Y, Song X, Wang CY, Chen C, Trudo SP, et al. Cruciferous vegetables have variable effects on biomarkers of systemic inflammation in a randomized controlled trial in healthy young adults. The Journal of nutrition. 2014; 144(11):1850-7.

257. Nadeem N, Woodside JV, Neville CE, McCall DO, McCance D, Edgar D, et al. Serum amyloid A-related inflammation is lowered by increased fruit and vegetable intake, while high-sensitive C-reactive protein, IL-6 and E-selectin remain unresponsive. The British journal of nutrition. 2014; 112(7):1129-36.

258. Zampelas A, Panagiotakos DB, Pitsavos C, Das UN, Chrysohoou C, Skoumas Y, et al. Fish consumption among healthy adults is associated with decreased levels of inflammatory markers related to cardiovascular disease: the ATTICA study. J Am Coll Cardiol. 2005; 46(1):120-4.

259. King DE, Egan BM, Geesey ME. Relation of dietary fat and fiber to elevation of C-reactive protein. The American journal of cardiology. 2003; 92(11):1335-9.

260. Madsen T, Skou HA, Hansen VE, Fog L, Christensen JH, Toft E, et al. C-reactive protein, dietary n-3 fatty acids, and the extent of coronary artery disease. The American journal of cardiology. 2001; 88(10):1139-42.

261. Hickling S, Hung J, Knuiman M, Divitini M, Beilby J. Are the associations between diet and C-reactive protein independent of obesity? Preventive medicine. 2008; 47(1):71-6.

262. Gump BB, MacKenzie JA, Dumas AK, Palmer CD, Parsons PJ, Segu ZM, et al. Fish consumption, low-level mercury, lipids, and inflammatory markers in children. Environmental research. 2012; 112:204-11.

263. Gump BB, Gabrikova E, Bendinskas K, Dumas AK, Palmer CD, Parsons PJ, et al. Low-level mercury in children: associations with sleep duration and cytokines TNF-alpha and IL-6. Environmental research. 2014; 134:228-32.

264. Ward RJ, Lallemand F, de Witte P. Influence of adolescent heavy session drinking on the systemic and brain innate immune system. Alcohol and alcoholism (Oxford, Oxfordshire). 2014; 49(2):193-7.

265. Costello EJ, Copeland WE, Shanahan L, Worthman CM, Angold A. C-Reactive Protein and substance use disorders in adolescence and early adulthood: A Prospective Analysis. Drug and alcohol dependence. 2013; 133(2):712-17.

266. Panagiotakos DB, Pitsavos CH, Zampelas AD, Chrysohoou CA, Stefanadis CI. Dairy products consumption is associated with decreased levels of inflammatory markers related to cardiovascular disease in apparently healthy adults: the ATTICA study. Journal of the American College of Nutrition. 2010; 29(4):357-64.

267. Labonte ME, Cyr A, Abdullah MM, Lepine MC, Vohl MC, Jones P, et al. Dairy product consumption has no impact on biomarkers of inflammation among men and women with low-grade systemic inflammation. The Journal of nutrition. 2014; 144(11):1760-7.

268. Bordoni A, Danesi F, Dardevet D, Dupont D, Fernandez AS, Gille D, et al. Dairy Products and Inflammation: A Review of the Clinical Evidence. Critical reviews in food science and nutrition. 2015:0.

269. Liu M, Gillis LJ, Persadie NR, Atkinson SA, Phillips SM, Timmons BW. Effects of Short-Term Exercise Training With and Without Milk Intake on Cardiometabolic and Inflammatory Adaptations in Obese Adolescents. Pediatric exercise science. 2015; 27(4):518-24.

270. Salas-Salvado J, Casas-Agustench P, Murphy MM, Lopez-Uriarte P, Bullo M. The effect of nuts on inflammation. Asia Pacific journal of clinical nutrition. 2008; 17 Suppl 1:333-6.

271. Jiang R, Jacobs DR, Jr., Mayer-Davis E, Szklo M, Herrington D, Jenny NS, et al. Nut and seed consumption and inflammatory markers in the multi-ethnic study of atherosclerosis. Am J Epidemiol. 2006; 163(3):222-31.

272. Ros E, Nunez I, Perez-Heras A, Serra M, Gilabert R, Casals E, et al. A walnut diet improves endothelial function in hypercholesterolemic subjects: a randomized crossover trial. Circulation. 2004; 109(13):1609-14.

273. Maranhao PA, Kraemer-Aguiar LG, de Oliveira CL, Kuschnir MC, Vieira YR, Souza MG, et al. Brazil nuts intake improves lipid profile, oxidative stress and microvascular function in obese adolescents: a randomized controlled trial. Nutrition & metabolism. 2011; 8(1):32.

274. Hu FB. Dietary pattern analysis: a new direction in nutritional epidemiology. Current opinion in lipidology. 2002; 13(1):3-9.

275. Barbaresko J, Koch M, Schulze MB, Nothlings U. Dietary pattern analysis and biomarkers of low-grade inflammation: a systematic literature review. Nutrition reviews. 2013; 71(8):511-27.

276. Ahluwalia N, Andreeva VA, Kesse-Guyot E, Hercberg S. Dietary patterns, inflammation and the metabolic syndrome. Diabetes & metabolism. 2013; 39(2):99-110.

277. Ko BJ, Park KH, Shin S, Zaichenko L, Davis CR, Crowell JA, et al. Diet quality and diet patterns in relation to circulating cardiometabolic biomarkers. Clinical nutrition (Edinburgh, Scotland). 2016; 35(2):484-90.

278. Schulze MB, Hoffmann K, Manson JE, Willett WC, Meigs JB, Weikert C, et al. Dietary pattern, inflammation, and incidence of type 2 diabetes in women. The American journal of clinical nutrition. 2005; 82(3):675-84; quiz 714-5.

279. Lopez-Garcia E, Schulze MB, Fung TT, Meigs JB, Rifai N, Manson JE, et al. Major dietary patterns are related to plasma concentrations of markers of inflammation and endothelial dysfunction. The American journal of clinical nutrition. 2004; 80(4):1029-35.

280. Nettleton JA, Steffen LM, Schulze MB, Jenny NS, Barr RG, Bertoni AG, et al. Associations between markers of subclinical atherosclerosis and dietary patterns derived by principal components analysis and reduced rank regression in the Multi-Ethnic Study of Atherosclerosis (MESA). The American journal of clinical nutrition. 2007; 85(6):1615-25.

281. Nettleton JA, Matijevic N, Follis JL, Folsom AR, Boerwinkle E. Associations between dietary patterns and flow cytometry-measured biomarkers of inflammation and cellular activation in the Atherosclerosis Risk in Communities (ARIC) Carotid Artery MRI Study. Atherosclerosis. 2010; 212(1):260-7.

282. Smidowicz A, Regula J. Effect of nutritional status and dietary patterns on human serum C-reactive protein and interleukin-6 concentrations. Advances in nutrition (Bethesda, Md). 2015; 6(6):738-47.

283. Fung TT, McCullough ML, Newby P, Manson JE, Meigs JB, Rifai N, et al. Diet-quality scores and plasma concentrations of markers of inflammation and endothelial dysfunction. The American journal of clinical nutrition. 2005; 82(1):163-73.

284. Dai J, Miller AH, Bremner JD, Goldberg J, Jones L, Shallenberger L, et al. Adherence to the mediterranean diet is inversely associated with circulating interleukin-6 among middle-aged men: a twin study. Circulation. 2008; 117(2):169-75.

285. Chrysohoou C, Panagiotakos DB, Pitsavos C, Das UN, Stefanadis C. Adherence to the Mediterranean diet attenuates inflammation and coagulation process in healthy adults: The ATTICA Study. J Am Coll Cardiol. 2004; 44(1):152-8.

286. Ambring A, Johansson M, Axelsen M, Gan L, Strandvik B, Friberg P. Mediterranean-inspired diet lowers the ratio of serum phospholipid n-6 to n-3 fatty acids, the number of leukocytes and platelets, and vascular endothelial growth factor in healthy subjects. The American journal of clinical nutrition. 2006; 83(3):575-81.

287. Esposito K, Marfella R, Ciotola M, Di Palo C, Giugliano F, Giugliano G, et al. Effect of a mediterranean-style diet on endothelial dysfunction and markers of vascular inflammation in the metabolic syndrome: a randomized trial. Jama. 2004; 292(12):1440-6.

288. Estruch R, Martinez-Gonzalez MA, Corella D, Salas-Salvado J, Ruiz-Gutierrez V, Covas MI, et al. Effects of a Mediterranean-style diet on cardiovascular risk factors: a randomized trial. Annals of internal medicine. 2006; 145(1):1-11.

289. Schwingshackl L, Hoffmann G. Mediterranean dietary pattern, inflammation and endothelial function: a systematic review and meta-analysis of intervention trials. Nutrition, metabolism, and cardiovascular diseases : NMCD. 2014; 24(9):929-39.

290. Guenther PM, Casavale KO, Reedy J, Kirkpatrick SI, Hiza HA, Kuczynski KJ, et al. Update of the Healthy Eating Index: HEI-2010. Journal of the Academy of Nutrition and Dietetics. 2013; 113(4):569-80.

291. Monfort-Pires M, Folchetti LD, Previdelli AN, Siqueira-Catania A, de Barros CR, Ferreira SR. Healthy Eating Index is associated with certain markers of inflammation and insulin resistance but not with lipid profile in individuals at cardiometabolic risk. Applied physiology, nutrition, and metabolism = Physiologie appliquee, nutrition et metabolisme. 2014; 39(4):497-502.

292. Arnold K, Andridge R, Logan A, Yee L, Lustberg M, Orchard T. Healthy Eating Index Scores are Associated with Inflammatory Markers in Breast Cancer Survivors. The FASEB Journal. 2015; 29(1 Supplement):LB267.

293. McCullough ML, Feskanich D, Stampfer MJ, Giovannucci EL, Rimm EB, Hu FB, et al. Diet quality and major chronic disease risk in men and women: moving toward improved dietary guidance. The American journal of clinical nutrition. 2002; 76(6):1261-71.

294. Akbaraly TN, Shipley MJ, Ferrie JE, Virtanen M, Lowe G, Hamer M, et al. Long-term Adherence to Healthy Dietary Guidelines and Chronic Inflammation in the Prospective Whitehall II Study(). The American Journal of Medicine. 2015; 128(2):152-60.e4.

295. Fung TT, Chiuve SE, McCullough ML, Rexrode KM, Logroscino G, Hu FB. Adherence to a DASH-style diet and risk of coronary heart disease and stroke in women. Archives of internal medicine. 2008; 168(7):713-20.

296. Azadbakht L, Borzuyee A, Esmaillzadeh A. Effects of Dietary Approaches to Stop Hypertension (DASH) Eating Plan on Inflammation and Liver Functional Tests among Type 2 Diabetic Patients. Zahedan Journal of Research in Medical Sciences. 2012; 14(2):9-15.

297. Saneei P, Hashemipour M, Kelishadi R, Esmaillzadeh A. The Dietary Approaches to Stop Hypertension (DASH) diet affects inflammation in childhood metabolic syndrome: a randomized cross-over clinical trial. Annals of nutrition & metabolism. 2014; 64(1):20-7.

298. Cavicchia PP, Steck SE, Hurley TG, Hussey JR, Ma Y, Ockene IS, et al. A new dietary inflammatory index predicts interval changes in serum high-sensitivity C-reactive protein. The Journal of nutrition. 2009; 139(12):2365-72.

299. Shivappa N, Steck SE, Hurley TG, Hussey JR, Hebert JR. Designing and developing a literature-derived, population-based dietary inflammatory index. Public health nutrition. 2014; 17(8):1689-96.

300. Xu H, Sjogren P, Arnlov J, Banerjee T, Cederholm T, Riserus U, et al. A proinflammatory diet is associated with systemic inflammation and reduced kidney function in elderly adults. The Journal of nutrition. 2015; 145(4):729-35.

301. van Woudenbergh GJ, Theofylaktopoulou D, Kuijsten A, Ferreira I, van Greevenbroek MM, van der Kallen CJ, et al. Adapted dietary inflammatory index and its association with a summary score for low-grade inflammation and markers of glucose metabolism: the Cohort study on Diabetes and Atherosclerosis Maastricht (CODAM) and the Hoorn study. The American journal of clinical nutrition. 2013; 98(6):1533-42.

302. Shivappa N, Steck SE, Hurley TG, Hussey JR, Ma Y, Ockene IS, et al. A population-based dietary inflammatory index predicts levels of C-reactive protein in the Seasonal Variation of Blood Cholesterol Study (SEASONS). Public health nutrition. 2014; 17(8):1825-33.

303. Tabung FK, Steck SE, Zhang J, Ma Y, Liese AD, Agalliu I, et al. Construct validation of the dietary inflammatory index among postmenopausal women. Annals of epidemiology. 2015

304. Ruiz-Canela M, Bes-Rastrollo M, Martinez-Gonzalez MA. The Role of Dietary Inflammatory Index in Cardiovascular Disease, Metabolic Syndrome and Mortality. International journal of molecular sciences. 2016; 17(8)

305. Shivappa N, Blair CK, Prizment AE, Jacobs DR, Jr., Steck SE, Hebert JR. Association between inflammatory potential of diet and mortality in the Iowa Women's Health study. European journal of nutrition. 2015

306. Shivappa N, Harris H, Wolk A, Hebert JR. Association between inflammatory potential of diet and mortality among women in the Swedish Mammography Cohort. European journal of nutrition. 2015

307. Galas A, Kulig J. Low-grade dietary-related inflammation and survival after colorectal cancer surgery. Journal of cancer research and clinical oncology. 2014; 140(9):1517-25.

308. Lu Y, Shivappa N, Lin Y, Lagergren J, Hebert JR. Diet-related inflammation and oesophageal cancer by histological type: a nationwide case-control study in Sweden. European journal of nutrition. 2015

309. Maisonneuve P, Shivappa N, Hebert JR, Bellomi M, Rampinelli C, Bertolotti R, et al. Dietary inflammatory index and risk of lung cancer and other respiratory conditions among heavy smokers in the COSMOS screening study. European journal of nutrition. 2015

310. Zamora-Ros R, Shivappa N, Steck SE, Canzian F, Landi S, Alonso MH, et al. Dietary inflammatory index and inflammatory gene interactions in relation to colorectal cancer risk in the Bellvitge colorectal cancer case-control study. Genes & nutrition. 2015; 10(1):447.

311. Shivappa N, Jackson MD, Bennett F, Hebert JR. Increased Dietary Inflammatory Index (DII) Is Associated With Increased Risk of Prostate Cancer in Jamaican Men. Nutrition and cancer. 2015:1-8.

312. Tabung FK, Steck SE, Ma Y, Liese AD, Zhang J, Caan B, et al. The association between dietary inflammatory index and risk of colorectal cancer among postmenopausal women: results from the Women's Health Initiative. Cancer causes & control : CCC. 2015; 26(3):399-408.

313. Shivappa N, Zucchetto A, Montella M, Serraino D, Steck SE, La Vecchia C, et al. Inflammatory potential of diet and risk of colorectal cancer: a case-control study from Italy. The British journal of nutrition. 2015; 114(1):152-8.

314. Shivappa N, Zucchetto A, Serraino D, Rossi M, La Vecchia C, Hebert JR. Dietary inflammatory index and risk of esophageal squamous cell cancer in a case-control study from Italy. Cancer causes & control : CCC. 2015

315. Wirth MD, Shivappa N, Steck SE, Hurley TG, Hebert JR. The dietary inflammatory index is associated with colorectal cancer in the National Institutes of Health-American Association of Retired Persons Diet and Health Study. The British journal of nutrition. 2015:1-9.

316. Shivappa N, Bosetti C, Zucchetto A, Montella M, Serraino D, La Vecchia C, et al. Association between dietary inflammatory index and prostate cancer among Italian men. The British journal of nutrition. 2014:1-6.

317. Shivappa N, Bosetti C, Zucchetto A, Serraino D, La Vecchia C, Hebert JR. Dietary inflammatory index and risk of pancreatic cancer in an Italian case-control study. The British journal of nutrition. 2014:1-7.

318. Ruiz-Canela M, Zazpe I, Shivappa N, Hebert JR, Sanchez-Tainta A, Corella D, et al. Dietary inflammatory index and anthropometric measures of obesity in a

population sample at high cardiovascular risk from the PREDIMED (PREvencion con DIeta MEDiterranea) trial. The British journal of nutrition. 2015; 113(6):984-95.

319. Garcia-Arellano A, Ramallal R, Ruiz-Canela M, Salas-Salvado J, Corella D, Shivappa N, et al. Dietary Inflammatory Index and Incidence of Cardiovascular Disease in the PREDIMED Study. Nutrients. 2015; 7(6):4124-38.

320. Wirth MD, Burch J, Shivappa N, Violanti JM, Burchfiel CM, Fekedulegn D, et al. Association of a dietary inflammatory index with inflammatory indices and metabolic syndrome among police officers. Journal of occupational and environmental medicine / American College of Occupational and Environmental Medicine. 2014; 56(9):986-9.

321. Wood LG, Shivappa N, Berthon BS, Gibson PG, Hebert JR. Dietary inflammatory index is related to asthma risk, lung function and systemic inflammation in asthma. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology. 2015; 45(1):177-83.

322. Sanchez-Villegas A, Ruiz-Canela M, de la Fuente-Arrillaga C, Gea A, Shivappa N, Hebert JR, et al. Dietary inflammatory index, cardiometabolic conditions and depression in the Seguimiento Universidad de Navarra cohort study. The British journal of nutrition. 2015:1-9.

323. Wirth MD, Hebert JR, Shivappa N, Hand GA, Hurley TG, Drenowatz C, et al. Anti-inflammatory Dietary Inflammatory Index scores are associated with healthier scores on other dietary indices. Nutrition research (New York, NY). 2016; 36(3):214-9.

324. Turner-McGrievy GM, Wirth MD, Shivappa N, Wingard EE, Fayad R, Wilcox S, et al. Randomization to plant-based dietary approaches leads to larger short-term improvements in Dietary Inflammatory Index scores and macronutrient intake compared with diets that contain meat. Nutrition research (New York, NY). 2015; 35(2):97-106.

325. Steck S, Shivappa N, Tabung F, Harmon B, Wirth M, Hurley T, et al. The dietary inflammatory index: a new tool for assessing diet quality based on inflammatory potential. Digest. 2014; 49:1-9.

326. Tabung FK, Smith-Warner SA, Chavarro JE, Wu K, Fuchs CS, Hu FB, et al. Development and Validation of an Empirical Dietary Inflammatory Index. The Journal of nutrition. 2016; 146(8):1560-70.

327. Huh J, Riggs NR, Spruijt-Metz D, Chou CP, Huang Z, Pentz M. Identifying patterns of eating and physical activity in children: a latent class analysis of obesity risk. Obesity (Silver Spring, Md). 2011; 19(3):652-8.

328. Lopes C, Aro A, Azevedo A, Ramos E, Barros H. Intake and adipose tissue composition of fatty acids and risk of myocardial infarction in a male Portuguese community sample. Journal of the American Dietetic Association. 2007; 107(2):276-86.

329. Silva D, Rego C, Guerra A. Characterization of food habits and comparative study between two methods of food assessment in adolescents. Rev Aliment Humana. 2004; 10:33–40.

330. Martins I, Porto A, Luísa O. Table Composition of Foods. Lisbon, Portugal: National Institute of Health Dr Ricardo Jorge; 2006.

331. Ferreira F, Graça M. Composition Table of Portuguese Food. 2nd ed. Lisbon, Portugal: National Institute of Health Dr Ricardo Jorge; 1985.

332. Goldberg GR, Black AE, Jebb SA, Cole TJ, Murgatroyd PR, Coward WA, et al. Critical evaluation of energy intake data using fundamental principles of energy physiology: 1. Derivation of cut-off limits to identify under-recording. European journal of clinical nutrition. 1991; 45(12):569-81.

333. Black AE. Critical evaluation of energy intake using the Goldberg cut-off for energy intake:basal metabolic rate. A practical guide to its calculation, use and limitations. International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity. 2000; 24(9):1119-30.

334. Schofield WN. Predicting basal metabolic rate, new standards and review of previous work. Human nutrition Clinical nutrition. 1985; 39 Suppl 1:5-41.

335. Trost SG, Ward DS, Moorehead SM, Watson PD, Riner W, Burke JR. Validity of the computer science and applications (CSA) activity monitor in children. Medicine and science in sports and exercise. 1998; 30(4):629-33.

336. Roark RA, Niederhauser VP. Fruit and vegetable intake: issues with definition and measurement. Public health nutrition. 2013; 16(1):2-7.

337. Fruit and vegetables for health: report of a Joint FAO/WHO Workshop, 1-3 September, 2004, Kobe, Japan. Fruit and vegetables for health: Report of a Joint FAO/WHO Workshop, 1-3 September, 2004, Kobe, Japan; 2005. World Health Organization.

338. Thompson FE, Willis GB, Thompson OM, Yaroch AL. The meaning of 'fruits' and 'vegetables'. Public health nutrition. 2011; 14(7):1222-8.

339. Rich C, Geraci M, Griffiths L, Sera F, Dezateux C, Cortina-Borja M. Quality Control Methods in Accelerometer Data Processing: Defining Minimum Wear Time. PloS one. 2013; 8(6):e67206.

340. Trost SG, Loprinzi PD, Moore R, Pfeiffer KA. Comparison of accelerometer cut points for predicting activity intensity in youth. Medicine and science in sports and exercise. 2011; 43(7):1360-8.

341. Cole TJ, Lobstein T. Extended international (IOTF) body mass index cut-offs for thinness, overweight and obesity. Pediatric obesity. 2012; 7(4):284-94.

342. Faria ER, Franceschini Sdo C, Peluzio Mdo C, Sant'Ana LF, Priore SE. Methodological and ethical aspects of the sexual maturation assessment in adolescents. Revista paulista de pediatria : orgao oficial da Sociedade de Pediatria de Sao Paulo. 2013; 31(3):398-405.

343. World Health Organization. Guidelines for Controlling and Monitoring the Tobacco Epidemic. Geneva: World Health Organization; 1998.

344. Enas EA, Senthilkumar A, Chennikkara H, Bjurlin MA. Prudent diet and preventive nutrition from pediatrics to geriatrics: current knowledge and practical recommendations. Indian heart journal. 2003; 55(4):310-38.

345. Kalogeropoulos N, Panagiotakos DB, Pitsavos C, Chrysohoou C, Rousinou G, Toutouza M, et al. Unsaturated fatty acids are inversely associated and n-6/n-3 ratios are positively related to inflammation and coagulation markers in plasma of apparently healthy adults. Clinica chimica acta; international journal of clinical chemistry. 2010; 411(7-8):584-91.

346. Poudel-Tandukar K, Nanri A, Matsushita Y, Sasaki S, Ohta M, Sato M, et al. Dietary intakes of alpha-linolenic and linoleic acids are inversely associated with serum C-reactive protein levels among Japanese men. Nutrition research (New York, NY). 2009; 29(6):363-70.

347. Navarro SL, Kantor ED, Song X, Milne GL, Lampe JW, Kratz M, et al. Factors Associated with Multiple Biomarkers of Systemic Inflammation. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2016; 25(3):521-31.

348. Kubota Y, Higashiyama A, Imano H, Sugiyama D, Kawamura K, Kadota A, et al. Serum Polyunsaturated Fatty Acid Composition and Serum High-Sensitivity C-Reactive Protein Levels in Healthy Japanese Residents: The KOBE Study. The journal of nutrition, health & aging. 2015; 19(7):719-28.

349. Sanchez-Moreno C, Cano MP, de Ancos B, Plaza L, Olmedilla B, Granado F, et al. Consumption of high-pressurized vegetable soup increases plasma vitamin C and decreases oxidative stress and inflammatory biomarkers in healthy humans. The Journal of nutrition. 2004; 134(11):3021-5.

350. Oliveira A, Maia B, Lopes C. Determinants of inadequate fruit and vegetable consumption amongst Portuguese adults. Journal of human nutrition and dietetics : the official journal of the British Dietetic Association. 2014; 27 Suppl 2:194-203.

351. Slavin JL, Lloyd B. Health benefits of fruits and vegetables. Advances in nutrition (Bethesda, Md). 2012; 3(4):506-16.

352. Krebs-Smith SM, Kantor LS. Choose a variety of fruits and vegetables daily: understanding the complexities. The Journal of nutrition. 2001; 131(2s-1):487s-501s.

353. Michels KB, Welch AA, Luben R, Bingham SA, Day NE. Measurement of Fruit and Vegetable Consumption with Diet Questionnaires and Implications for Analyses and Interpretation. American journal of epidemiology. 2005; 161(10):987-94.

354. Bhupathiraju SN, Wedick NM, Pan A, Manson JE, Rexrode KM, Willett WC, et al. Quantity and variety in fruit and vegetable intake and risk of coronary heart disease. The American journal of clinical nutrition. 2013; 98(6):1514-23.

355. Buchner FL, Bueno-de-Mesquita HB, Ros MM, Overvad K, Dahm CC, Hansen L, et al. Variety in fruit and vegetable consumption and the risk of lung cancer in the European prospective investigation into cancer and nutrition. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2010; 19(9):2278-86.

356. Buchner FL, Bueno-de-Mesquita HB, Ros MM, Kampman E, Egevad L, Overvad K, et al. Variety in vegetable and fruit consumption and risk of bladder cancer in the European Prospective Investigation into Cancer and Nutrition. International journal of cancer. 2011; 128(12):2971-9.

357. Agudo A. Measuring intake of fruit and vegetables. FAO/WHO Workshop on Fruit and Vegetable for Health, 1-3 September 2004, Kobe, Japan. World Health Organization; 2005. Disponível em: http://www.who.int/dietphysicalactivity/publications/f&v_intake_measurement.pdf.

358. Raynor HA, Epstein LH. Dietary variety, energy regulation, and obesity. Psychological bulletin. 2001; 127(3):325-41.

359. Mattes R. Soup and satiety. Physiology & behavior. 2005; 83(5):739-47.

360. Shivappa N, Hebert JR, Rietzschel ER, De Buyzere ML, Langlois M, Debruyne E, et al. Associations between dietary inflammatory index and inflammatory markers in the Asklepios Study. The British journal of nutrition. 2015; 113(4):665-71.

361. Connaughton R, Kirwan A, McMorrow A, McGillicuddy F, Lithander F, Roche H. Combination of Anti-Inflammatory Nutrients shows Synergistic Potential at Modulating Pro-Inflammatory Cytokines in an In Vitro Setting. The FASEB Journal. 2015; 29(1 Supplement):LB234.